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# JOURNAL OF EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY

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# JOURNAL OF EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY

## Harold Barnes, Founder Editor

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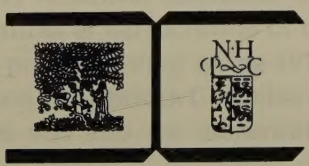

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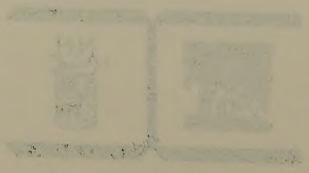

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# DEVELOPMENT OF THE SAND-DWELLING MEIOBENTHIC HARPACTICOID COPEPOD *HUNTEMANNIA JADENSIS* Poppe IN THE LABORATORY[1]

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**Abstract:** *Huntemannia jadensis* Poppe, a common intertidal copepod in coastal waters of the northwestern United States and British Columbia, Canada ranges in length from 0.1 mm at birth to 1.3 mm (adult female) and 1.5 mm for the larger of two male morphs. Body length alone is not a reliable indicator of the stage of development for this species. All six naupliar and the first two copepodite stages are interstitial, but older stages are burrowers. Individual sand grains were grasped with antennae, first maxillae, and caudal rami for browsing attached diatoms. Males of both morphs clasped immature females up to several days before females molted to the adult stage and were inseminated. In cultures with daily addition of benthic diatoms, *H. jadensis* grew from egg to adult in 50 days at both 15 and 20 °C, 66 days at 12 °C, and 95 days at 8 °C. Development never progressed past the naupliar stages at 4 °C. Females lived for more than a year in culture, and a maximum of four broods per female was produced with broods 3 to 5 wk apart. A single observation of generation time (egg to egg) of 167 days was made at 8 °C. Laboratory egg mortality ranged from 17 to 83% per brood. Naupliar mortality was also very high, but copepodites suffered less than 25% mortality within each stage. Survivorship to maturity in the laboratory was highest at 8 °C. Laboratory mortality patterns were similar to those observed in field populations. Growth was so slow and cultures so difficult to maintain that *H. jadensis* is not recommended for future laboratory study of burrowing or interstitial harpacticoid copepods.

## INTRODUCTION

The importance of meiobenthos, and especially harpacticoid copepods, in detrital food webs of coastal and estuarine waters has been demonstrated with increasing frequency over the past decade (Coull & Bell, 1979). Benthic harpacticoid copepods form a large fraction of the diets of several juvenile bottom-feeding fishes (e.g., Bregnballe, 1961; Odum & Heald, 1972; Feller and Kaczynski, 1975), and close coupling between predatory juvenile salmon and their harpacticoid prey has been documented by Sibert (1979) in British Columbia waters. These same juvenile salmon contained large quantities of harpacticoids in their diet and grew at a rate of 6% of their body weight per day during spring 1975 (Healy, 1979). The most abundant harpacticoid copepod in the British Columbia study area was *Huntemannia jadensis* Poppe. This species was also the numerically dominant sand-dwelling

[1] Contribution No. 1148 from the Department of Oceanography, University of Washington, Seattle, Washington 98195.

harpacticoid on three intertidal beaches in Puget Sound sampled by Feller (in press). Natural populations of *H. jadensis* occur over a broad range of tidal elevations in abundances ranging seasonally from 10 to over 1000 per 10 $cm^2$; they had either one or two generations per year, and comprised an average of 26% (range 5–80%) of the total harpacticoid assemblage over an 18-month period at one intertidal sampling station in Puget Sound, Washington, U.S.A. (Feller, in press).

Attempts to model the ecological role of meiobenthic organisms in the marine environment have been frustrated by a lack of basic information on the energy flow, life history, and reproductive biology of these small animals (e.g., Steele, 1974). Thus *Huntemannia jadensis* was reared in the laboratory to estimate its critical reproductive and growth parameters over a range of temperatures experienced by natural intertidal populations.

Laboratory cultures should simulate the organism's natural environment as closely as possible. Although the most difficult factor to mimic for a burrowing and/or interstitial copepod like *H. jadensis* is the tidal flux of water through the intertidal sediments, no attempt was made to model such a regime in this study. Instead, factors typically encountered in the culture of small crustaceans were initially addressed: substratum requirements, food quantity and quality, salinity and temperature tolerance, size and type of culture vessel, cannibalism, and the possibility of batch culture. Details of these preliminary observations are outlined in Feller (1977). Final culture methods are described below.

## Materials and Methods

Gravid females of *H. jadensis* were collected from the mean lower low water (MLLW) tidal level on three similar sand beaches in Puget Sound (Golden Gardens, Richmond Beach, and Mukilteo; see Feller, in press, for site descriptions) at various times to initiate cultures. Most cultures were started during the spring of 1973 and 1974. Of 200 initiated, 50 cultures were unsuccessful either because eggs did not hatch or because all nauplii died before molting to the first copepodite stage. Data from cultures lost before any individuals had molted to the adult stage have been utilized where possible.

### CULTURING PROCEDURES

Live gravid females were collected by scooping the top centimeter of sand into jars containing sea water. Single females were then picked from the sediment and isolated in plastic Petri dishes, 35 × 10 mm, which had been soaked in sea water for 1 day. A 2- to 3-mm layer of fresh sand from the MLLW tidal level at the Golden Gardens collection area was added to the Petri dishes after it had been picked free of other meiofauna except ciliates and small nematodes which were impossible to remove completely. A few of these organisms were present in the culture dishes at all times.

The small Petri dishes were half-filled with sea water (*S*, 29‰) and covered. Six to eight of the small dishes were placed into a larger plastic Petri dish, 100 × 20 mm, and covered. The larger dishes were stacked in environmental chambers under continuous dim fluorescent light at 8, 12 and 15 °C. Cultures at 4 °C were kept inside a continuously dark refrigerator, and cultures at 20 °C were kept away from sunlight on a laboratory bench.

*Census*

Cultures were checked daily under a dissecting microscope. After the eggs hatched, the female was removed to a fresh culture dish for further observation. The daily routine consisted of counting each developmental stage, removing fecal material, dead animals and molted exoskeletons if present, and adding fresh sea water and food. Each culture dish was censused at approximately the same time each day, so that all observations were subject to an error of $\pm\frac{1}{2}$ day. Since this error affected all observations equally, it has been ignored.

*Food and substratum*

Fresh food for the animals was obtained by vigorously shaking about 20 $cm^3$ of fresh surface sand from Golden Gardens. Detached benthic diatoms were decanted into a shallow Petri dish and unwanted metazoans removed before adding the diatoms in suspension evenly over the substratum in the culture dishes during the daily census. This new food material quickly settled onto sand grains in the dish and was browsed by the copepods. The copepods always had food in their guts when observed during the daily census. All individuals in each culture dish were transferred to a newly prepared dish at ≈2-wk intervals.

Benthic diatoms present in the food included *Navicula* spp., *Nitzschia paradoxa*, other *Nitzschia* spp., *Biddulphia laevis*, *Rhizosolenia* spp., *Melosira* spp., *Pinnularia* spp., *Triceratium* spp., *Tropidoneis* sp., *Skeletonema costatum*, *Coscinodiscus* spp., and other unidentified centric and pennate diatoms. Intact large cells were not ingested by the naupliar and copepodite stages of *H. jadensis*. Feeding concentrated on the smaller naviculoid cells, although *Skeletonema* and fecal material were also ingested. Direct utilization of bacterial and detrital material could not be determined, but all stages of the copepod appeared to graze upon it.

*Molt size*

During daily maintenance and censusing of the 8 and 12 °C cultures, freshly cast exoskeletons were removed and their lengths, from tip of rostrum to base of caudal rami, measured to the nearest 0.01 mm. Measurements from several cultures at each temperature were pooled and length–frequency histograms constructed. Naupliar molts were not measured, as these usually could not be found or separated from the

culture debris. Stage and size of nauplii were determined on freshly preserved individuals from viable cultures.

*Development*

Eggs developed internally and were visible as two dark green rows in oviducts oriented longitudinally beneath the dorsal exoskeleton. All eggs in the pair of oviducts were extruded together into a pair of ovisacs which extended laterally from beneath the genital segment. Freshly-extruded, dark blue-green eggs became progressively lighter colored each day in the ovisacs.

Egg development time (from extrusion to hatching) could not be determined for gravid females collected in the field because it was not possible to know how long they had carried their eggs. Direct determinations of egg development time were made by isolating females with internal eggs until their eggs were extruded into ovisacs. This time was designated as $t_0$ in estimating development time of eggs. The original dark blue-green egg color persisted for a day or two, so estimates of $t_0$ for cultures utilizing females already carrying ovisacs with eggs the same color as freshly extruded eggs are in error by the same amount. Hence egg development times reported are minimum estimates.

Individual naupliar stages could not be accurately distinguished on live individuals under the dissecting microscope, so development time from hatching to first appearance of C I's (copepodite stage I) was recorded for nauplii as a group. With no way to mark or tag individuals, I did not know exactly how long an individual had remained in the previous stage. However, since copepodites from the same brood usually molted to the next stage over a period of about 2 days, development times could be estimated by recording the times of first appearance of each stage from $t_0$. Averages of times for all cultures within a temperature allowed derivation of residence times within each copepodite stage by subtracting the total time to stage $i$ from time to stage $i + 1$. Direct measures of development times for several isolated individuals differed from those estimated by the above method by only 1 or 2 days at most.

Estimates of development times for the life stages of *H. jadensis* cultured from the three areas were combined and averaged for each temperature. Estimates of residence time within successive stages were summed to give estimates of times from hatching to maturity.

*Fecundity*

The number of nauplii hatching from each female's first brood in the laboratory was recorded according to the female's area of collection, time of isolation, and culture temperature. The number of nauplii hatching from successive broods of females which produced more than one brood in the laboratory was also recorded.

*Mortality*

Eggs carried by females in laboratory culture could not be counted directly because of movement by the animals. To compute laboratory egg mortality, it was assumed that freshly isolated females carried the same number of eggs as females from the field population at the time females were collected and isolated in culture. The percentage of inviable eggs per first laboratory brood was calculated by month for each area as:

$$\% \text{ inviable} = \frac{(\text{no. eggs/field brood}) - (\text{no. eggs hatching/lab. brood})}{(\text{no./field brood})} (100),$$

with no. eggs/field brood from Feller (in press).

Survivorship curves were constructed for the laboratory populations by combining results from each area at each experimental temperature. Stage-specific mortality, or the number of a stage dying as a percentage of the total number of individuals which had successfully molted to that stage, was estimated by summing results over all areas at each temperature.

### BEHAVIORAL OBSERVATIONS

Direct observations of locomotory mechanisms, phototactic responses, feeding, copulation, and molting were made routinely during the daily census.

## RESULTS

### STAGE IDENTIFICATION

Development consisted of six naupliar and six copepodite stages. The last (sixth) copepodite stage was the adult. Nauplii ranged in length from 0.10 mm at birth to $\approx$0.20 mm in the sixth stage. Copepodites, variable in length because of the telescoping ability of the free thoracic segments, were easily identified by the addition of one free thoracic segment with each molt. First-stage copepodites (C I's) had three free body segments, and adults had eight.

Males may first be distinguished from females in the fourth copepodite stage by a rudimentary knob or clasper on the distal segment of their antennules and by a long spine on each caudal ramus. The clasper became functional in the fifth copepodite stage, but it was not fully developed until the adult stage. Males exhibited dimorphism in the C V and adult stages, with the small adult morph about the same size and length as adult females or slightly smaller. The prehensile claspers and antennules of the large adult morph were disproportionately larger than those of the small morph, but both morphs clasped females with equal proficiency. Male dimorphism has also been reported for one species of planktonic harpacticoid (Haq, 1973).

Length–frequency distribution modes were distinct for the first three copepodite stages, but modes for C-IV and C-V males and females overlapped. Length measurements of individuals from field samples encompassed the range of exoskeleton lengths measured for animals reared in the laboratory (Table I). The existence of

TABLE I

Length ranges (mm) for *Huntemannia jadensis* derived from measurements of freshly molted exoskeletons recovered from laboratory cultures[a], preserved specimens from the field[b], or preserved culture specimens[c]; length measured from tip of rostrum to insertion of caudal rami on anal segment.

| Stage | Length |
|---|---|
| Nauplii[c] | 0.10–0.20 |
| Copepodite I[a] | 0.29–0.39 |
| Copepodite II[a] | 0.39–0.48 |
| Copepodite III[a] | 0.49–0.61 |
| Copepodite IV[a] (males and females) | 0.60–0.75 |
| Copepodite V females[a,b] | 0.75–0.84 |
| Copepodite V males (small morph)[a,b] | 0.75–0.85 |
| (large morph)[a,b] | 0.80–1.04 |
| Adult females[b] | 0.83–1.30 |
| Adult males (small morph)[b] | 0.80–1.15 |
| (large morph)[b] | 1.10–1.55 |

large and small morphs of C-V males contributed to the variability in length observed for laboratory-reared indiviuals of stage C V. Lengths of adult *H. jadensis* reported by Lang (1948) (males 0.8 to 0.9 mm and females 0.9 and 1.0 mm) and Sars (1911) (females 0.96 mm) should not be regarded as diagnostic for the species. The wide range of lengths within a stage was undoubtedly due to telescopic lengthening and shortening of the body upon preservation.

## BEHAVIOR

The naupliar and first two copepodite stages assumed an interstitial habit, crawling through spaces between sand grains. Other stages were burrowers and displaced sand grains as they moved. Locomotion was normally effected by the legs, but it was common to see animals use their antennae and caudal rami to push and pull themselves through the sand. They moved awkwardly over the surface of sand grains, and swimming motions were jerky. They could be induced to swim for short periods by agitation with a probe. None of the stages before C I were observed to swim. Typical behavior upon agitation of all copepodite and adult stages was immediate curling of the body upon itself or around a sand grain with prolonged

motionless periods. Phototactic response was variable, with some individuals exhibiting both positive and negative phototaxes.

Feeding behavior involved grasping a sand grain with antennae, first maxillae, and caudal rami. The grain of sand was manipulated and rotated as the animal grazed upon attached algae and bacteria. If the grain was too large to be rotated, the animal maintained a purchase on its surface while browsing. First-stage nauplii fed soon after hatching. Filter-feeding currents were not produced by any developmental stage.

Males developed slightly faster than females, presumably as a reproductive adaptation to ensure their presence when females molt to the adult stage. Females were clasped by their caudal rami with the male's prehensile claspers. This posture was maintained for periods of a few minutes to several days. If a C-V female was clasped, the male usually maintained its hold until the female made her final molt. At this time the male released the molted exoskeleton and immediately reclasped the female. The molting process took $\approx 2$ min. Spermatophore transfer took place as male and female interlocked with genital segments juxtaposed. Apparently females were fertilized only once, as females which had been fertilized were never again seen clasped in laboratory cultures. Single inseminations have been reported to produce multiple broods in other harpacticoids (Johnson & Olson, 1948). Precocious clasping, also previously reported for other harpacticoids (Williams, 1907; Willey, 1930) was common, as C-V males clasped adult females. Adult males of both morphs were observed clasping C IV, C V, and adult females and occasionally other males, albeit briefly.

## DEVELOPMENT TIMES

Estimates of development times for *H. jadensis* from each area were combined and averaged for each experimental temperature. Although eggs hatched in the 4 °C cultures, nauplii never reached stage C I; at higher temperatures development times decreased with increasing temperature (Table II). Between 15 and 20 °C the growth rate stabilized or declined, as animals raised at 20 °C took slightly longer to develop. However, since many more observations of development times were made at 15 °C, and the 20 °C results were quite variable, this difference may not be real. It took *H. jadensis* an average of 95 days to reach the adult stages at 8 °C, 66 days at 12 °C, and 50 days at both 15 and 20 °C.

Only one culture produced a new generation in the laboratory. A female was clasped by a sibling male (large morph) 92 days after their birth at 8 °C. Eggs first appeared in the oviducts on Day 107 and were extruded into ovisacs on Day 153. The eggs hatched on Day 167, with nine nauplii appearing. Therefore, the only measure of generation time (from hatch to hatch) for *H. jadensis* in laboratory culture was 167 days. Several other females clasped by sibling males extruded eggs in culture, but none of the eggs hatched. For 10 females at 8 °C, clasping occurred an average

TABLE II

Development times (days) for separate stages of *Huntemannia jadensis* grown in laboratory cultures at five temperatures: data for all three areas combined; Naup. includes all naupliar stages combined; *N*, number of observations.

| Temp. (°C) | | Egg | Naup. | C I | C II | C III | C IV | C V | Total |
|---|---|---|---|---|---|---|---|---|---|
| 4 | $\bar{x}$ | 11.6 | | | | | | | |
| | SD | 5.2 | | | | | | | |
| | *N* | 5 | | | | | | | |
| 8 | $\bar{x}$ | 11.7 | 37.6 | 9.6 | 7.6 | 8.4 | 8.4 | 11.9 | 95.2 |
| | SD | 5.3 | 6.9 | 3.1 | 1.8 | 2.4 | 2.3 | 2.3 | |
| | *N* | 62 | 181 | 157 | 37 | 129 | 109 | 115 | |
| 12 | $\bar{x}$ | 9.7 | 25.9 | 5.3 | 4.8 | 5.7 | 6.5 | 8.4 | 66.3 |
| | SD | 5.0 | 9.2 | 1.3 | 1.3 | 1.3 | 2.1 | 2.9 | |
| | *N* | 81 | 277 | 72 | 56 | 46 | 40 | 28 | |
| 15 | $\bar{x}$ | 7.3 | 18.2 | 4.5 | 3.5 | 4.0 | 5.1 | 6.1 | 48.7 |
| | SD | 2.3 | 2.8 | 1.0 | 1.3 | 1.2 | 1.4 | 1.6 | |
| | *N* | 11 | 82 | 69 | 67 | 65 | 59 | 56 | |
| 20 | $\bar{x}$ | 4.7 | 18.6 | 6.3 | 4.8 | 5.7 | 5.5 | 4.0 | 49.6 |
| | SD | 1.9 | 5.4 | 3.4 | 1.8 | 2.5 | 4.9 | 2.0 | |
| | *N* | 32 | 94 | 6 | 5 | 6 | 2 | 3 | |

of 85 days after birth, with egg extrusion averaging 81 days after clasping and insemination. Two females at 15 °C were clasped by sibling males 58 days after birth, and neither extruded eggs before they died.

## FECUNDITY

In most cultures, females died soon after being isolated from their newly hatched brood. A few females at each temperature produced additional broods after isolation, and three females at 8 °C and two at 15 °C produced four broods each in culture. It is unknown whether four broods is the maximum which females can produce, because multiple broods resulted from fertilizations in the field before females were isolated in culture. The time between successive broods was highly variable within a given temperature, but generally 3 to 4 wk elapsed between broods (Table III).

The number of nauplii which hatched from an individual female's first laboratory brood varied greatly and averaged 14.1 for all females and ranged from 4.6 per brood at 4 °C to 15.5 per brood at 12 °C (Table IV). Because so few females produced more than one brood at each experimental temperature, it is difficult to determine whether hatching success remained constant for successive broods. The number of nauplii hatching from second, third, and fourth broods was variable but averaged between 11 and 12 (Table IV).

TABLE III

Time (days) between successive broods produced by *Huntemannia jadensis* at five temperatures: *N*, number of observations.

| Temp. (°C) | Brood number | No. days eggs in oviducts | | | No. days eggs in ovisacs | | | Interbrood period (days) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $\bar{x}$ | SD | *N* | $\bar{x}$ | SD | *N* | $\bar{x}$ | SD | *N* |
| 4 | 2 | 14.0 | 9.4 | 4 | 1.5 | 0.7 | 2 | 7.5 | 0.7 | 2 |
| | 3 | 39.0[a] | | 1 | | | | | | |
| 8 | 2 | 25.7 | 7.8 | 11 | 13.4 | 2.2 | 8 | 35.2 | 4.9 | 8 |
| | 3 | 16.5 | 8.6 | 6 | 13.2 | 1.8 | 5 | 27.6 | 7.2 | 5 |
| | 4 | 14.7[a] | 4.0 | 3 | | | | | | |
| 12 | 2 | 24.3 | 19.9 | 6 | 10.5 | 7.3 | 6 | 34.8 | 18.2 | 6 |
| 15 | 2 | 21.3 | 15.4 | 6 | 7.0 | 1.7 | 5 | 26.6 | 17.6 | 5 |
| | 3 | 12.5 | 10.7 | 4 | 7.8 | 3.0 | 4 | 20.2 | 9.8 | 4 |
| | 4 | 14.3 | 16.7 | 3 | 5.3 | 3.1 | 3 | 26.0 | 0.0 | 2 |
| 20 | 2 | 26.0 | 4.2 | 2 | 1.0[b] | | 2 | | | |

[a] Females died with eggs still in oviducts.
[b] Females died with eggs still in ovisacs.

TABLE IV

Number of nauplii hatching from successive laboratory broods of *Huntemannia jadensis* at five temperatures: *N*, number of observations.

| Temp. (°C) | Brood number | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | | 2 | | | 3 | | | 4 | | |
| | $\bar{x}$ | SD | *N* | $\bar{x}$ | SD | *N* | $\bar{x}$ | SD | *N* | $\bar{x}$ | SD | *N* |
| 4 | 4.6 | 3.6 | 5 | 3.5 | 0.7 | 2 | – | – | – | – | – | – |
| 8 | 15.2 | 8.8 | 40 | 9.8 | 4.4 | 8 | 13.0 | 3.6 | 5 | – | – | – |
| 12 | 15.5 | 6.7 | 68 | 13.5 | 6.5 | 6 | – | – | – | – | – | – |
| 15 | 8.1 | 6.3 | 11 | 14.4 | 4.2 | 5 | 11.0 | 6.9 | 4 | 11.0 | 9.9 | 2 |
| 20 | 13.1 | 5.5 | 26 | – | – | – | – | – | – | – | – | – |
| Average | 14.1 | 7.5 | 150 | 11.0 | 5.9 | 21 | 12.1 | 5.0 | 9 | 11.0 | 9.9 | 2 |

## MORTALITY AND SURVIVORSHIP

All cultures at all experimental temperatures were grouped to estimate egg mortalities for each month over the study in which cultures were initiated. Egg mortality was high in cultures from each area, and from 17 to 83% of all eggs extruded by females in their first laboratory brood died or failed to hatch (Table V). It is unknown whether eggs were ever resorbed while in the oviducts.

Survivorship of individuals reared in laboratory culture was computed by com-

TABLE V

Percent mortality of first-brood eggs in laboratory cultures averaged by month over all experimental temperatures for three areas.

| Month | Mukilteo | Richmond Beach | Golden Gardens |
|---|---|---|---|
| 1973 | | | |
| January | 54.3 | – | – |
| February | 38.9 | – | – |
| March | – | – | – |
| April | 50.5 | – | – |
| May | 16.8 | – | – |
| June | – | – | – |
| July | 40.8 | – | – |
| August | – | – | – |
| September | 68.2 | – | – |
| October | 76.0 | – | – |
| November | 38.1 | 51.1 | – |
| December | – | 42.5 | – |
| 1974 | | | |
| January | – | 20.7 | 39.7 |
| February | 43.0 | – | – |
| March | – | 61.8 | 28.8 |
| April | 55.2 | 81.4 | 67.5 |
| May | 58.6 | 80.3 | 71.0 |
| June | 77.5 | 83.0 | 55.3 |

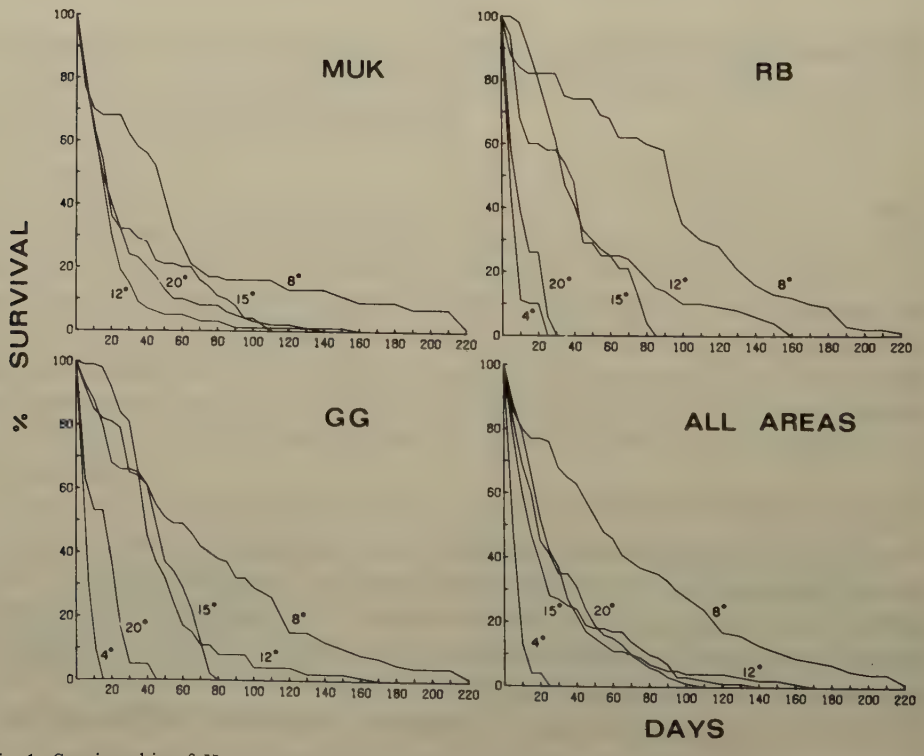


Fig. 1. Survivorship of *Huntemannia jadensis* from each study area according to experimental temperature for laboratory cultures: MUK, Mukilteo; RB, Richmond Beach; GG, Golden Gardens.

bining results from within each area at each experimental temperature (Fig. 1). Survivorship was highest in 8 °C cultures, and in most cultures females lived more than 220 days. A few females lived over a year in the laboratory at 8 °C, and two females lived 400 days before the cultures were terminated.

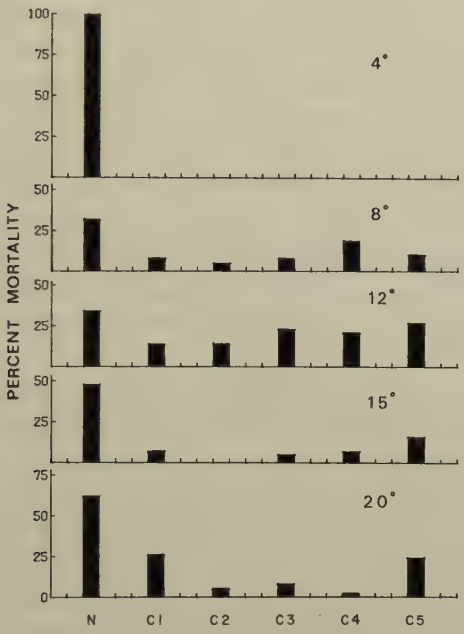


Fig. 2. Mortality in laboratory cultures of *Huntemannia jadensis* averaged over all study areas at five experimental temperatures: mortality of each stage equals proportion of total number of individuals which molted to that stage.

Mortality of individual stages, or the number of individuals dying within a stage as a percentage of the number which had successfully molted to that stage, was estimated by summing results for all areas. Naupliar mortality was higher than for any other stage at all experimental temperatures, ranging from 100% at 4 °C to 32% at 8 °C (Fig. 2). Generally, <25% of any copepodite stage failed to reach the next stage of development.

## Discussion

Information on the growth and development of individual harpacticoid species adapted for burrowing into and through the sediment or for existence in the interstitial environment is scarce. Rao (1967) raised *Arenopontia indica* in watch glasses with sand and described its successive developmental stages, but little methodological information was provided. *A. indica* apparently grew from egg to adult in 25 to

30 days at an unspecified temperature. Harris (1972) was unsuccessful in attempts to rear interstitial species. Hardy (1978) described a convenient method for rearing sand-dwellers and reported that several species have been successfully raised in the laboratory; no data on growth and development were provided, however. The scarcity of information on interstitial harpacticoids is probably due to the difficulties inherent in rearing them and the relative ease with which phytal and epibenthic forms may be reared (Goswami, 1977; Hoppenheit, 1978). The fast-growing epibenthic forms may be grown under a variety of conditions and will utilize a wide variety of different foods (see Table 1 in Omori, 1973; Table 1 in Smol & Heip, 1974; Rieper, 1978; Kahan, 1979). Epibenthic harpacticoids reared by Muus (1967), Coull & Dudley (1976), and Harris (1977) took from 2 to 6 wk to develop from egg to adult. Phytal harpacticoids develop even faster (see Table 6 in Rosenfield & Coull, 1974). Intrinsic rates of population increase for most laboratory populations of harpacticoids fall in the range 0.04 to 0.30 per day, depending on culture conditions (Volkmann-Rocco & Fava, 1969; Heip, 1972; Heip & Smol, 1976; Zurlini *et al.*, 1978).

Because only one observation of generation time was made in laboratory cultures, insufficient data exist to compute instantaneous rates of increase ($r$) for the culture populations of *Huntemannia jadensis*. However, from data on fecundity and generation time reported here, a rough estimate is possible by assuming that the net reproductive rate per female ($R_0$) is 15 individuals and that generation time ($T$) is 150 days. Using the relation $r = \ln R_0/T$ from Fenchel (1968), $r$ is $0.018 \cdot \text{day}^{-1}$. This value and others derived by small changes in the above assumptions regarding the magnitudes of $R_0$ and $T$ are similar to those derived from field abundance estimates (Feller, in press).

Fenchel (1968) derived a log–log relationship between $r$ and generation time for many types of organisms from virus to man. The point on his line for harpacticoid copepods was taken from sparse data for rapidly-growing and primarily epibenthic species (Muus, 1967). The generation time of *H. jadensis* is an order of magnitude longer than predicted by Fenchel's relationship (hundreds vs. tens of days). Based on considerations of body size alone, development times for *H. jadensis* are also considerably longer than expected. Compared with other similarly sized harpacticoids, *H. jadensis* took nearly twice as long to reach maturity. Some of this difference may undoubtedly be ascribed to latitudinal differences and differences in culture techniques employed by other workers, but, in general, it appears that phytal, epibenthic, and tide-pool species grow faster and have shorter generation times than burrowing or interstitial forms. If other meiobenthic harpacticoids are found to have similarly long developmental and/or generation times, Fenchel's generalization for the taxon may not be valid.

Under the variety of culture temperatures, collection sites, presumed excess natural food conditions, and varied light regimes at each temperature investigated, *H. jadensis* grew and developed at rates comparable to those deduced from field

collections at these same sites. It took between 50 and 95 days to develop from egg to adult in the laboratory (Table II), and the only estimate of generation time was 167 days at 8 °C. Successive broods appeared at approximately 4-wk intervals (Table III). Given that development times decrease with increasing temperature, these laboratory growth rate estimates compare favorably with the developmental sequence observed in the field where interstitial temperatures can fluctuate as much as 5 °C daily and 10 °C seasonally. From the time when maximum numbers of gravid females were present, it took approximately 5 or 6 months before adult females increased in abundance (Feller, in press).

Causes of laboratory mortality are difficult to define. Great care was taken to remove excess detrital material which was observed to cause naupliar mortality in preliminary culture experiments. Females were removed from culture dishes soon after their broods hatched as a precaution against potential (but unobserved) cannibalism, and there were no known predators present in the culture vessels. Mortality of eggs and nauplii accounted for the largest amount of mortality in all laboratory cultures (Table V, Figs. 1 and 2), and similar patterns of mortality were observed in field populations of *H. jadensis* (Feller, in press). Copepodite mortality was comparatively low and relatively constant between stages in both laboratory and field populations of *H. jadensis*.

The ability to culture any organism in the laboratory is advantageous for a variety of reasons, e.g., genetic research, pollution studies, aquaculture. However, in view of the difficulties encountered in developing a successful culturing technique for *H. jadensis* (it took nearly a year of preliminary experiments), I cannot recommend this species for future laboratory studies. My attempts at mass culture were failures. Use of improved culture techniques such as devised by Hardy (1978) may prove useful in future studies of sand-dwelling species, but if other harpacticoids with similar life-modes grow as slowly as *H. jadensis*, the amount of time necessary to perform multigeneration studies is simply too great under current funding regimes. Fast-growing species (e.g., *Tisbe* or *Tigriopus*) should be used instead.

## Acknowledgements

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# EFFECTS OF FOOD AVAILABILITY ON THE VALVE MOVEMENTS AND FEEDING BEHAVIOR OF JUVENILE *CRASSOSTREA VIRGINICA* (Gmelin). II. FEEDING RATES AND BEHAVIOR

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**Abstract:** The effects of changes in availability of algal food on the feeding behavior of *Crassostrea virginica* (Gmelin) were investigated. Individual oysters were subjected to three conditions: (1) continuously fed, the algal suspension maintained at a constant concentration (50,000 cells · $ml^{-1}$) by an automatic turbidostat, (2) discontinuously fed, where oysters were alternately fed with the turbidostat for 12 h and unfed in filtered sea water for 12 h, and (3) batch fed, in which oysters were given a specific daily algal ration which was subsequently grazed. Valve movements of the oysters were concurrently recorded and rates of algae removal measured.

Rates of removal of algae from suspension by oysters maintained under constant algal concentration varied from hour to hour. However, when cumulative ration removed per activity period (valves open) was plotted as a function of the duration of the activity period, the relationship was linear with slope = 1.02. Time-averaged rates of algal removal were thus constant for all oysters and consistent among different individuals. Average rates of algal removal by oysters fed continuously and discontinuously showed no statistical difference. Consequently, daily rations cleared by oysters fed discontinuously were approximately half those cleared by oysters fed continuously.

Based on the observed relation between food availability and the valve movements of oysters, a post-ingestive mechanism of detection of organic seston level is suggested.

## INTRODUCTION

Effects of the availability of food (algal suspension or natural seston), as influenced by changes in its concentration, on the feeding rates of filter-feeding bivalves have been well documented (Davids, 1964; Winter, 1969; 1973; Ali, 1970; Foster-Smith, 1975a; Schulte, 1975; Widdows *et al.*, 1979). Generally, filtration rate (volume of water completely cleared of particles per unit time) decreases with increasing concentration, the net effect being a regulation of food uptake whereby the amount of particles filtered per unit time remains relatively constant (for review see Winter, 1978). Regulation of seston removal is imprecise, however, because rates of particle removal have been found in some cases to increase with increasing concentration (Thompson & Bayne, 1974; Langton *et al.*, 1977) and can also be affected by pseudofeces production (Winter, 1969; Foster-Smith, 1975a).

Methods used in the measurement of seston removal rates by bivalves have differed from study to study. In many investigations, rates were measured by the exponential decrease in algal concentration over time. The accuracy of this method has been criticized, however, and many studies using this method were conducted

over short periods of time so that long-term feeding behavior could not be assessed. As an alternative method, Winter (1969, 1973) and Riisgard & Mohlenberg (1979) used an automatic turbidostat, which maintained algal suspensions at constant concentration, to measure algae removal rates by bivalves. Although these studies were conducted over long periods of time (up to 3 days), the clearance rates were determined for groups of animals and not for individuals. Thus, any short-term variation in algae removal by an individual animal could not have been detected.

The effect of food availability, as influenced by its presence or absence, on the feeding rates of bivalves has not been studied. The effects of the presence–absence of food on the valve-movement patterns of activity and quiescence in the oyster *Crassostrea virginica* (Gmelin) have been reported by Higgins (1980). In the present paper, the effects of food availability, in particular with regard to its presence or absence, on the feeding rates of individual *C. virginica* are investigated. In addition, long-term and short-term feeding behavior under the conditions of constant concentration of algal suspension and some relationships between feeding and valve movements in oysters are discussed.

## Materials and Methods

Maintenance of oysters prior to experimentation, experimental conditions to which they were subjected, and the apparatus used in the experiments have been described elsewhere (Higgins, 1980). Individual animals were used in all experiments. Oysters maintained under the continuous, discontinuous, and batch-fed regimes were used in this study. The concentration of algal suspension (*Isochrysis galbana* Parke) was maintained at a constant level (50,000 ± 5000 cells/ml) by the turbidostat for the continuously and discontinuously fed oysters. For batch-fed oysters, the concentration of suspension decreased with time. Initial algal concentration for batch-fed oysters given the maximum ration was 200,000 cells/ml; for those given the 40% ration, 80,000 cells/ml.

The rate of removal of algae from suspension by oysters in the continuous and discontinuous regimes was measured by the turbidostat. Removal rate was calculated as $R = nftc$, where $R$ = algae removal rate (cells/h), $n$ = number of additions of reservoir algae per hour (from the event recorder data), $f$ = flow rate of the bellows pump (ml/sec), $t$ = pumping time per addition of reservoir algae (sec), and $c$ = concentration of the reservoir suspension (cells/ml). Preliminary tests were conducted to determine suitable values for $c$, $t$, and $f$.

To measure the removal of algae by batch-fed oysters throughout the course of feeding, the concentration of suspension was measured with a Coulter counter (model $Z_B$) fitted with a 70-$\mu$m aperture. Counts were made at the beginning and end of each 24-h feeding period and at intervals in between.

Valve movements of oysters were continuously recorded using a strain gauge

apparatus (Higgins, 1980) in order to determine the relation between feeding and valve movements. Oysters were cemented (Eastman 910 adhesive) onto a cuvette pedestal, which served to collect pseudofeces as they were sloughed off the oyster's anterior end. Although some pseudofeces were resuspended, most could be collected by this method. Pseudofeces were removed by pipette every 24 h, filtered through a glass fiber filter, washed with 0.5 N ammonium formate, dried, and weighed. The number of cells shunted to pseudofeces was estimated by assuming a cellular dry weight of *I. galbana* of 23.5 pg/cell (Epifanio & Ewart, 1977). Daily ingested ration for batch-fed oysters was determined by subtracting pseudofeces produced from the ration cleared from suspension.

## Results

Rates of removal of algae from suspension by oysters maintained under constant algal cell concentration (continuous and discontinuous regimes) varied considerably from hour to hour within any given period of activity (valves open). This variation in feeding rate is shown graphically in Fig. 1 and is representative for all oysters tested. Rates as a whole ranged from 0 to $23.8 \times 10^7$ cells $\cdot$ h$^{-1}$. Average rate of algae removal by continuously fed oysters was $9.3 \pm 4.50 \times 10^7$ cells $\cdot$ animal$^{-1}$ $\cdot$ h$^{-1}$, and for discontinously fed oysters, $10.6 \pm 7.32 \times 10^7$ cells $\cdot$ animal$^{-1}$ $\cdot$ h$^{-1}$. These rates were not statistically different (ANOVA, 0.1 probability level). Pseudofeces production for continuously fed oysters averaged 5.1% of the total algal ration (no. cells/3-day experimental period) removed from suspension.

For oysters fed under the batch regimes, the total algal rations removed per 24-h feeding period varied. Daily rations cleared and ingested depended on the algal ration given. Oysters fed the maximum ration cleared an average of $8.5 \pm 1.69 \times 10^8$ cells $\cdot$ day$^{-1}$ and ingested $7.9 \pm 1.77 \times 10^8$ cells $\cdot$ day$^{-1}$. Oysters given the 40% ration cleared $3.3 \pm 0.52 \times 10^8$ cells $\cdot$ day$^{-1}$ and ingested $3.1 \pm 0.59$ cells $\cdot$ day$^{-1}$.

Typical algae removal by batch fed oysters is shown in Fig. 2. Times at which the first closure of the valves (after introduction of the daily ration) occurred are indicated. It is apparent that filtration of algae stopped for periods of 1–2 h before valve closure was initiated. This occurred in most of the individuals tested. The concentration of suspension at which valve closure occurred for the first time was determined for each oyster. For both batch regimes combined, this concentration averaged 11,000 cells $\cdot$ ml$^{-1}$, and for the maximum and 40% ration groups separately, the average concentrations were 16,000 cells $\cdot$ ml$^{-1}$ and 7000 cells $\cdot$ ml$^{-1}$, respectively.

Characteristic valve movements of oysters fed under the continuous, discontinuous and batch regimes have been described (Higgins, 1980).

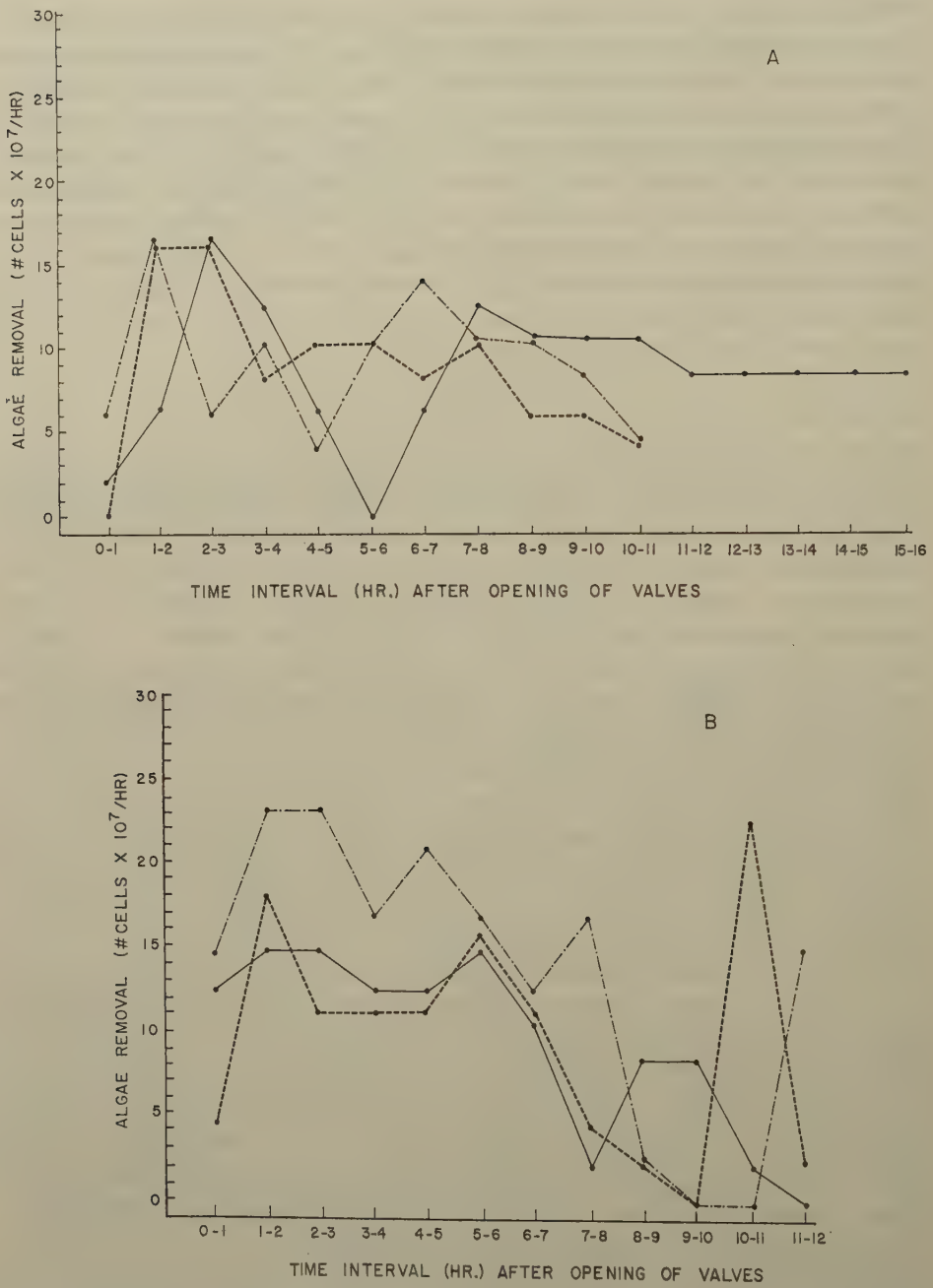


Fig. 1. Representative algal removal by *Crassostrea virginica* as a function of time, showing hourly variation in rates: A, continuous regime; B, discontinuous regime; each figure represents data for three different activity periods within the same individual oyster, part of a 3-day continuous record of feeding activity.

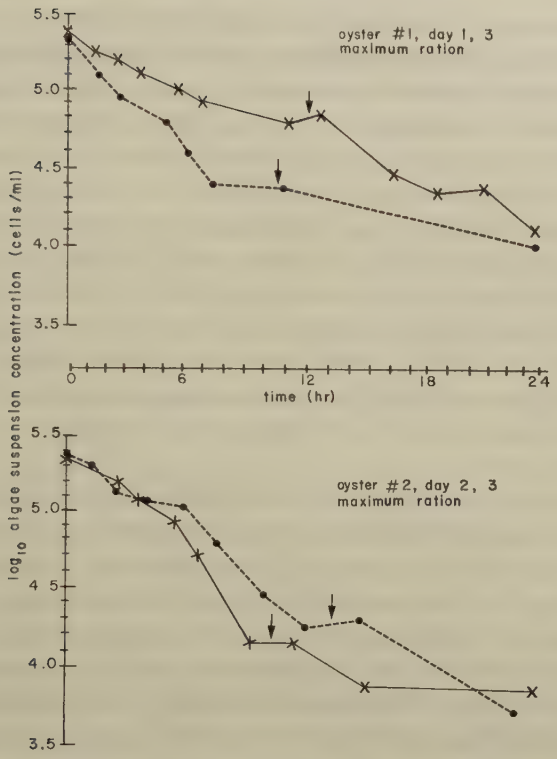


Fig. 2. Typical algae clearance over 24 h for batch-fed oysters, demonstrating the cessation of filtration prior to first closure (indicated by arrows).

## Discussion

### FEEDING RATES

Although activity periods (valves open) define when an oyster is capable of feeding, oysters did not feed at a constant rate throughout their periods of activity. Clearance rates by *Crassostrea virginica* maintained under constant algal cell concentration (continuous and discontinuous regimes) varied from hour to hour, and at times oysters appeared to filter no algae from suspension while the valves remained open (Fig. 1). Rates of algal removal also appeared to be slightly higher at the beginning of the activity periods, and this was more pronounced for the discontinuously fed oysters. Oysters normally began filtering algae immediately after opening their valves, and for the continuously and discontinuously fed oysters filtration continued up until the time of valve closure. Oysters in the batch regime often stopped filtering algae from suspension for the terminal 1–2 h prior to closure (Fig. 2). Variable rates of filtration of algae from suspension by individual *C. virginica* have also been

reported by Palmer (pers. obs.), and have been found in *Mytilus edulis* (groups of animals) by Winter (1973) and *Argopecten irradians* (individuals) by Palmer (pers. obs.). Thus, in addition to periodic opening and closure of the valves, there apparently is periodic variation in the intensity of feeding within periods of activity.

Variation in the clearance rate of *Crassostrea virginica* could be due to changes in the rate of pumping, alteration of the efficiency of particle retention of the gill, or both these factors in combination. Jørgensen (1976), studying excised gills, found that two species of *Crassostrea* (*C. cucullata* and *C. lugubris*) and many other species of bivalves could alter the retentive state of the gill by contractions of the gill muscles. Variable, periodic pumping (measured directly) has been demonstrated in *C. virginica* (Loosanoff & Engle, 1947), *Scrobicularia plana* (Earll, 1975), and *Arctica islandica* (Brand & Taylor, 1974). This evidence suggests that both processes could be operating in *Crassostrea virginica* to bring about varying rates of algal removal during periods of activity.

When oysters were subjected to the continuous feeding regime, they were exposed to an essentially unlimited availability of food. Whatever algae were removed from suspension were automatically replaced by the turbidostat. Under these conditions, satiation of feeding, i.e., the removal of a ration of algae that either satisfies the animal's physiological requirements for food or mechanically saturates its digestive organs, may possibly occur. The attainment of nutritive satiation could be accompanied by valve closure. To determine whether or not this occurs, the relation between the duration of periods of activity and the cumulative ration cleared within each period of activity was examined for the continuously fed oysters (Fig. 3). It is evident that cumulative algal ration removed per activity period is directly dependent on the duration of activity. Greater rations were cleared by oysters remaining open for longer periods of time. The relation in Fig. 3 shows that the algal rations cleared by oysters before quiescence is initiated are inconsistent for different activity periods, ranging from 0 to almost $4 \times 10^9$ cells. Thus, for the species of algae used in the present study and for the particular concentration (50,000 cells $\cdot$ ml$^{-1}$), it can be concluded that either satiation does not occur or that satiation is not the stimulus that brings about valve closure in oysters. However, a concentration of 50,000 cells $\cdot$ ml$^{-1}$ may be too low for satiation to be possible. Higher concentrations would increase the availability of food at any given time, which could allow greater numbers of cells to be cleared per unit time. Consequently, the gut could fill more rapidly, increasing the possibility of satiation. Experimentation at higher algal concentrations is necessary before any conclusions on satiation feeding and its relation to valve movements can be derived.

Because cumulative ration cleared per activity period was linearly proportional to the duration of activity (slope = 1.02, Fig. 3), it would indicate that the average rates of algae removal are relatively constant and are consistent among different individuals in the continuous regime. The possible factors controlling the rate of algal cell removal are: (1) the concentration of suspension (Thompson & Bayne,

1974; Langton *et al.*, 1977), or (2) mechanical limitation of the biomass (volume)/time capable of being processed by the organs of feeding and/or digestion (Williams, 1978). Either of these factors may explain why time-averaged rates of algae removal by oysters were constant under the conditions of constant suspension concentration.

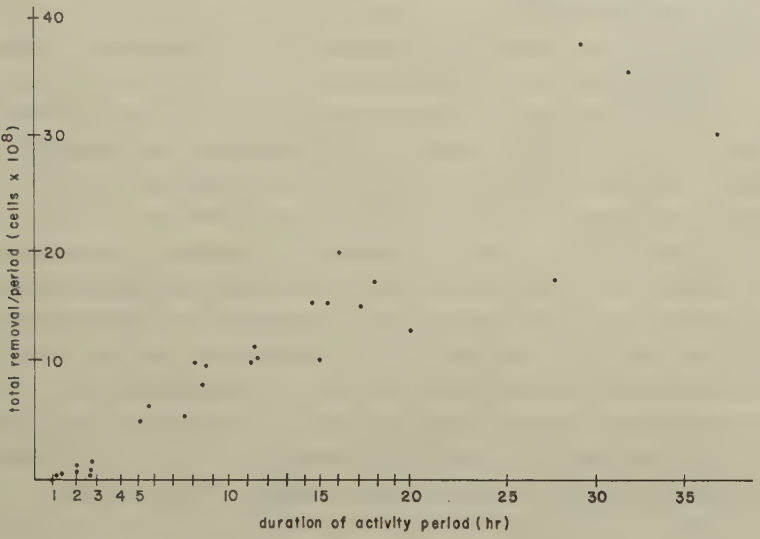


Fig. 3. Relation between the cumulative algal ration removed during an activity period and the duration of the activity period for the continuously fed oysters: linear regression for the data: $\hat{y} = 1.02x - 1.26$, $r^2 = 0.95$, $n = 27$.

Oysters fed continuously and discontinously showed similar average rates of algae clearance ($9.3 \times 10^7$ cells $\cdot$ h$^{-1}$ and $10.6 \times 10^7$ cells $\cdot$ h$^{-1}$, respectively). Thus, although oysters in the discontinuous regime were unfed 50% of the time, the average rate of feeding when food was available was much the same as that for oysters fed 100% of the time. Consequently, total algal ration removed by discontinuously fed oysters is limited by the duration of time the animals are exposed to food. The average daily algal ration cleared by continuously fed oysters was $2.2 \times 10^9$ cells $\cdot$ 24 h$^{-1}$, whereas that for discontinuously fed oysters was $1.2 \times 10^9$ cells $\cdot$ 24 h$^{-1}$ or 54.5% that of the continuously fed group. Thus, there appears to be no mechanism of regulation of food intake to compensate for temporal variation in the availability of food. *C. virginica* seems to regulate its rate of algae clearance on the basis of the immediate ambient conditions of algae availability, without apparent regard to prior (12 h, at least) feeding conditions. Time-dependent availability of food may consequently affect growth rates in bivalves by delimiting the total ration of food ingested by the animal and/or influencing the digestive process (e.g., digestive tubule synchrony) and efficiency of assimilation. Experiments with *C. gigas* (Langton & McKay, 1976)

and *Mytilus edulis* (Winter & Langton, 1975) have shown that exposure to different continuous and discontinuous feeding regimes does affect the rate of growth. However, these studies disagree in regard to which regime brings about most efficient growth. Mariculture-related research concerning optimum diets for bivalves could be further augmented by study of the effects of different feeding regimes on gross growth and growth efficiency.

Palmer (pers. obs.) measured average feeding rates of adult *Crassostrea virginica* fed *Isochrysis galbana* at different concentrations. At a concentration of 50,000 cells $\cdot$ ml$^{-1}$, the average feeding rate was found to be $7 \times 10^7$ cells $\cdot$ h$^{-1}$ $\cdot$ g dry wt$^{-1}$ (4 mg $\cdot$ h$^{-1}$ $\cdot$ g dry wt$^{-1}$) or about $2 \times 10^6$ cells $\cdot$ h$^{-1}$ $\cdot$ g whole wet wt$^{-1}$. This agrees reasonably well with the feeding rates of juvenile oysters in the present study (about $10^7$ cells $\cdot$ h$^{-1}$ $\cdot$ g whole wet wt$^{-1}$). Differences could be due to the different sizes of the experimental animals.

The average daily ration removed by batch-fed oysters given the maximum ration was $8.5 \times 10^8$ cells $\cdot$ day$^{-1}$. Oysters fed continuously with the turbidostat cleared a daily ration 2.6 times greater than this, and those fed discontinuously cleared a ration 1.4 times greater. Thus, *Crassostrea virginica* is capable of removing daily rations of *Isochrysis galbana* greater than the "maximum" rations estimated by Epifanio & Ewart (1977). The differences in daily ration cleared by oysters under the turbidostat and batch conditions are likely due to differences in total available food and to differences in suspension concentration and constancy of concentration.

## DETECTION OF SESTON

The introduction of food to unfed oysters (in filtered sea water) was found to induce prolonged activity and to bring about the initiation of an activity period if an oyster were quiescent. The reaction of *Crassostrea virginica* to the food stimulus is almost immediate. When algae were introduced to the discontinuously fed oysters after the 12-h unfed period, valve opening usually occurred within 15–30 min (Higgins, 1980). This reaction was also observed in the batch fed animals after introduction of the daily ration (Higgins, 1980). Similarly, Salanki (1966) observed that many species of bivalves, when closed, would immediately open when fresh sea water was introduced. Seston contained in the sea water might have caused this stimulation of activity. Davids (1964) and Thompson & Bayne (1972) noted that the introduction of food to starved *Mytilus edulis* immediately stimulated increased rates of ventilation and $O_2$ consumption.

The reaction of quiescent bivalves to the presence of suspended food could be the result of the animal's ability to detect the ambient seston level without completely opening its valves and ventilating its gills. Perhaps during quiescence the animal maintains a slight gape which exposes the edge of the mantle, but not the mantle cavity, to the medium. Neuroreceptors in the mantle might detect suspended matter either mechanically or chemically, thus providing the stimulus for opening of the

valves and the initiation of pumping and feeding activity. There is also some indication that a threshold concentration of seston may be required to stimulate activity in quiescent bivalves (Wilson & Seed, 1974).

Evidence suggests that *Crassostrea virginica* possesses some sensory mechanism for the detection and assessment of ambient nutritive conditions during activity. Although sudden exposure to unfed conditions always brought about valve closure in oysters under the discontinuous regime, this reaction was never immediate. Upon removal of the algae from suspension and its replacement with filtered sea water, oysters continued to remain open for periods of 2 to 3 h before closing for the first time (Higgins, 1980). If the sensory mechanism were located on the gills, the abrupt change in seston availability would be quickly detected as water passed over the gill, and one would expect the closure response to be more immediate. Thus, a gill-located mechanism may not be present in *C. virginica*. An alternative mechanism of detection may be post-ingestive in nature. If no nutritionally useful particles enter the stomach and are digested and absorbed, eventually the physiological consequences of starvation would become evident. Physiological starvation would indicate that food is not present in the medium, and the animal might close its valves in response. This mechanism would not result in immediate closure, because food already in the gut at the time the algae are removed would take a few hours to be digested (Mathers, 1972), and the physiological effects of starvation would not be apparent until the gut was cleared. A post-ingestive/digestive mechanism of detection of nutritive conditions, as opposed to a gill-located mechanism, may be of greater advantage to *C. virginica*. This species typically lives in turbid water (Galtsoff, 1964) in which seston contains a high proportion of inorganic matter. Recently, feeding experiments have shown that suspension-feeding bivalves filter and ingest organic and inorganic particles non-selectively (Foster-Smith, 1975b; Widdows *et al.*, 1979). Consequently, total ingestion of nutritionally useful seston is reduced by dilution by inorganic suspended matter. If the quantitative determination of food availability depended solely on a gill-located neuroreceptor, then oysters might remain open and continue to filter material from suspension even if the seston contained a high proportion of inorganics. Under these conditions, food energy removed from suspension might not sufficiently meet the relatively high energy demand for the mechanical cost (i.e., pumping and sorting) of feeding (Bayne *et al.*, 1976; Bayne & Scullard, 1977) and would eventually result in a negative energy balance. If, however, the detection of and reaction to the ambient food level depended on a post-ingestive mechanism, the ingestion of seston of high inorganic content would bring about starvation; and the animal would respond by closing its valves after a short time and undergoing standard (i.e., non-feeding) metabolism (see Thompson & Bayne, 1974; Bayne & Scullard, 1977). This would conserve the oyster's expenditure of energy due to the mechanical cost of feeding.

Behavior of batch-fed oysters during conditions of low algal cell density (i.e., several hours after the ration was introduced) was similar to the unfed behavior

of the discontinuously fed animals. Thus, it appears that not only does the absence of seston bring about the frequent opening/closure behavior, but low levels of seston will induce this behavior as well. It is possible that there exists a minimum threshold seston concentration below which sufficient food cannot be obtained and quiescence would be initiated (see Wilson & Seed, 1974). For suspensions of *Isochrysis galbana*, the concentration at which batch-fed oysters closed for the first time (after introduction of the ration) averaged 11,000 cells/ml. Either the algal concentrations to which the oysters were exposed or the ration of algae ingested prior to exposure to low concentrations of suspension might possibly affect the threshold concentration necessary to induce closure. Oysters fed the maximum ration, which were subjected to higher concentrations of suspension and ingested a greater ration of algae, closed at an average concentration of 16,000 cells $\cdot$ ml$^{-1}$. Oysters fed the 40% ration closed on the average of 7000 cells $\cdot$ ml$^{-1}$. The fact that oysters fed the different rations initiated first closure at different concentrations could account for the lack of difference in the percentage time open found between the two batch-fed groups (Higgins, 1980).

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# TEMPERATURE-INFLUENCED VARIATIONS IN SPECIATION AND CHEMICAL COMPOSITION OF MARINE PHYTOPLANKTON IN OUTDOOR MASS CULTURES[1]

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**Abstract:** Between September, 1976 and July, 1977 *Phaeodactylum tricornutum* Bohlin was replaced as the dominant species by *Skeletonema costatum* (Grev.) Cleve as temperatures fell below 10 °C in the fall in an outdoor pond supplied with a mixture of waste water and sea water. *Phaeodactylum tricornutum* returned in the spring as the major species when temperatures rose above 10 °C. In an adjacent pond in which only nitrogen and phosphorus were added in excess, however, *P. tricornutum* dominated throughout the entire study period even though the temperature varied between 0 and 25 °C. We suspected that the difference in species dominance in the two ponds occurred because *Skeletonema costatum* requires silicon, which was present in sufficient quantities only in the waste-water-enriched pond, whereas *Phaeodactylum tricornutum* does not have a specific requirement for this nutrient. The cellular chemical composition of *P. tricornutum* varied in a U-shaped fashion with changing temperature: minimum values for the cellular carbon, nitrogen, and chlorophyll contents were displayed at 15–20 °C and maximum values at 3 and 25 °C. Both the cellular carbon: nitrogen and carbon: chlorophyll ratios by weight were invariant with changing temperatures at ≈6 : 1 and 50 : 1 respectively, indicating nutrient saturation. Only under conditions of nutrient saturation, which can be established in various ways, can the influence of temperature on phytoplankton physiology be separated from nutrient-related factors.

## INTRODUCTION

Temperature plays a major role in influencing the outcome of competition between phytoplankton species in both enriched laboratory and outdoor mass cultures (Goldman & Ryther, 1976; Goldman, 1977a). For example, from 6 yr of data collection at Woods Hole Oceanographic Institution where large scale outdoor cultures were maintained, a pattern of seasonal shifts in species was observed. *Skeletonema costatum* dominated at temperatures below 10 °C, *Phaeodactylum tricornutum* between ≈10–23 °C, and assorted other species (e.g., *Nitzschia closterium*, *Amphora* sp., *Stichococcus* sp., *Nannochloris* sp.) above 23 °C (Goldman & Ryther, 1975, 1976; D'Elia *et al.*, 1977). In laboratory studies in which temperature was isolated as the major variable, it was shown that this parameter was more important than sunlight in influencing competition among species (Goldman & Ryther, 1976; Goldman, 1977a).

Temperature also has a dramatic effect on the chemical composition of phytoplankton, leading to large increases in various cellular constituents at the temperature

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extremes at which a particular species may grow (Goldman, 1977a, b). With outdoor cultures this phenomenon is difficult to observe because temperature-related competition prevents one particular species from dominating over a wide enough temperature range to allow sufficient data collection. Yet, numerous species such as *Phaeodactylum tricornutum* are eurythermal and will grow over a wide temperature range under laboratory conditions when not in competition with other species (Goldman & Ryther, 1976).

In this study we report one set of outdoor experiments (winter–spring, 1977) in which an unusual set of circumstances led to the dominance of *P. tricornutum* Bohlin over a wide temperature range (0–25 °C) so that it was possible to measure changes in cellular constituents that were attributable to temperature variations.

## Materials and Methods

### EXPERIMENTAL PONDS

Continuous cultures of marine phytoplankton were maintained in two 135,000-l ponds between September, 1976 and July, 1977. The ponds, each 15 $m^2$ and $\approx 1$ m deep, were constructed of earth and sand with a 0.5-mm thick polyvinyl chloride liner. Mixing was accomplished by circulating the contents of each pond with one 1100- and two 150-$l \cdot min^{-1}$ capacity centrifugal pumps (see Goldman & Ryther, 1976 for pond details). A mixture of $\approx 15$–$20\%$ secondarily treated waste water and $\approx 80$–$85\%$ sea water was supplied to one pond, and enriched sea water consisting of $NO_3^-$ and $PO_4^{3-}$ additions to natural sea water (in proportions comparable to a mixture of $\approx 15$–$30\%$ waste water and $\approx 70$–$85\%$ sea water) was supplied to the other pond. Dilution rates (= flow rate/culture volume) of $0.12$–$0.25 \cdot day^{-1}$ were maintained throughout the study. The waste water originated from the activated sludge treatment plant at Wareham, Massachusetts and typically contained $\approx 1.7$–$2.0$ mg at. $\cdot l^{-1}$ of total inorganic nitrogen ($NH_4^+ + NO_2^- + NO_3^-$) and $\approx 0.25$–$0.40$ mg at. $\cdot l^{-1}$ of $PO_4^{3-}$ (Goldman, 1976a; D'Elia *et al.*, 1977).

No attempt was made to inoculate a particular phytoplankton species into the ponds initially. The sea water entering the ponds was sand filtered to 20 $\mu$m to remove zooplankton and larger organisms, but indigenous phytoplankton were added continuously. Typically, only one species dominated at a given time.

### POND SAMPLING AND MEASUREMENTS

Pond sampling was carried out two to three times weekly from December, 1976 through July, 1977 in the waste-water–sea-water pond, and from February through June, 1977 in the pond supplied with enriched sea water. Measurements were made for cell identifications and counts (Spencer Bright-line hemocytometer), particulate carbon and nitrogen (Perkin Elmer 240 elemental analyzer), and chlorophyll *a*

(Strickland & Parsons, 1972). Pond temperatures were recorded daily on maximum–minimum thermometers and solar irradiation was measured continuously with an Eppley 6-90 pyranometer.

## Results

### SEASONAL SPECIES COMPOSITION

In the pond supplied with the nutrient–sea-water mixture a virtual monoculture of *P. tricornutum* persisted from September, 1976 through July, 1977. Pond temperatures varied in the range 0–25 °C during this time. In the waste-water–sea-water pond *P. tricornutum* dominated until early November, 1976 when it was replaced quickly by *Skeletonema costatum* (Grev.) Cleve (Fig. 1C). This species change coincided with a seasonal drop in pond temperatures to $<10$ °C (Fig. 1B). *S. cos-*

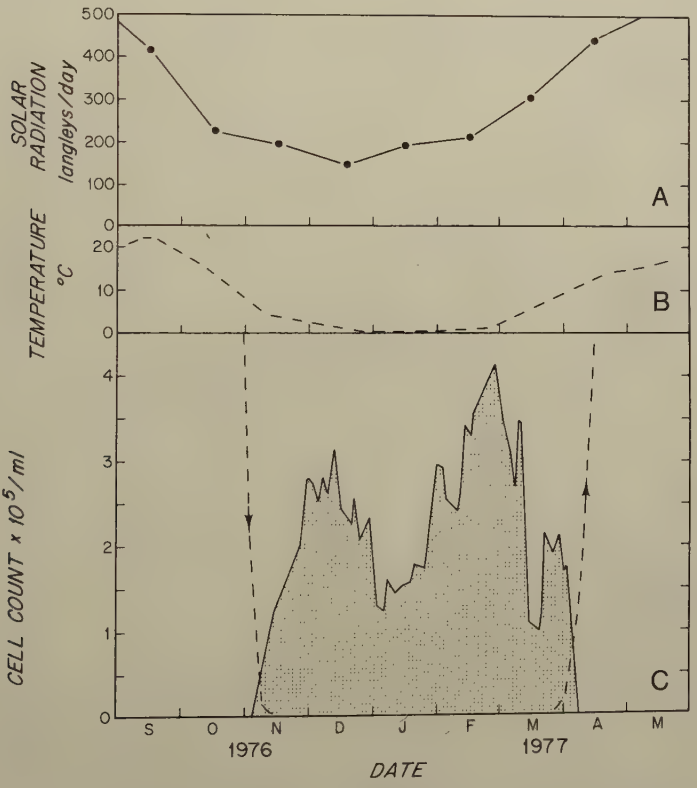


Fig. 1. Seasonal variations in environmental parameters and phytoplankton speciation in outdoor continuous culture at Woods Hole, Massachusetts, during the period September, 1976–May, 1977: A, solar radiation; B, temperature; C, cell count; dashed lines, *Phaeodactylum tricornutum*; shaded area, *Skeletonema costatum*.

*tatum* completely dominated until early April, 1977 when it was once again rapidly replaced by *Phaeodactylum tricornutum* as pond temperatures were rising to 10 °C (Fig. 1C). The latter species was dominant until the pond culture was terminated in June, 1977.

## CELLULAR CARBON AND NITROGEN CHANGES

Sampling for cellular constituents in the pond containing *P. tricornutum* did not begin until mid-February, 1977 when temperatures had risen to ≈3 °C. During the sampling period U-shaped variations in the cellular carbon (Fig. 2B) and nitrogen (Fig. 2C) contents of this species with temperature were measured. Maximum cellular contents (≈20 pg C · cell$^{-1}$ and ≈4 pg N · cell$^{-1}$) occurred at both the low (3–4 °C) and high (24–25 °C) temperatures, and minimum values (≈6 pg C · cell$^{-1}$

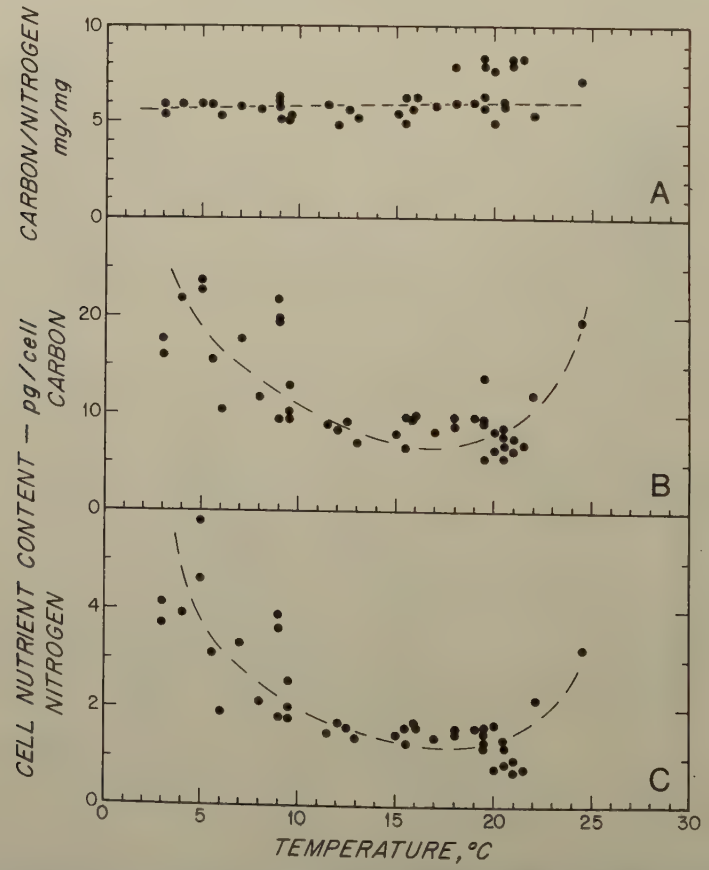


Fig. 2. Temperature-dependent variations in cellular carbon and nitrogen for *Phaeodactylum tricornutum* grown in outdoor continuous cultures: A, carbon : nitrogen ratio; B, cellular carbon; C, cellular nitrogen; dashed lines in Figs. 2–4 were drawn by eye and are meant to represent trends only.

and ≈ 1.2 pg N · cell $^{-1}$) were reached between 15–20 °C. The constant carbon : nitrogen ratio of 5.8 : 1 (Fig. 2A) is indicative that changes in both cellular constituents were always proportional.

In contrast, it was impossible to observe more than a few degrees change (0 → 5 °C) when *Skeletonema costatum* completely dominated the pond. Both the cellular carbon and nitrogen contents of this species appeared to remain virtually constant at ≈ 30 pg C · cell $^{-1}$ and ≈ 5 pg N · cell $^{-1}$ during this period (Table I).

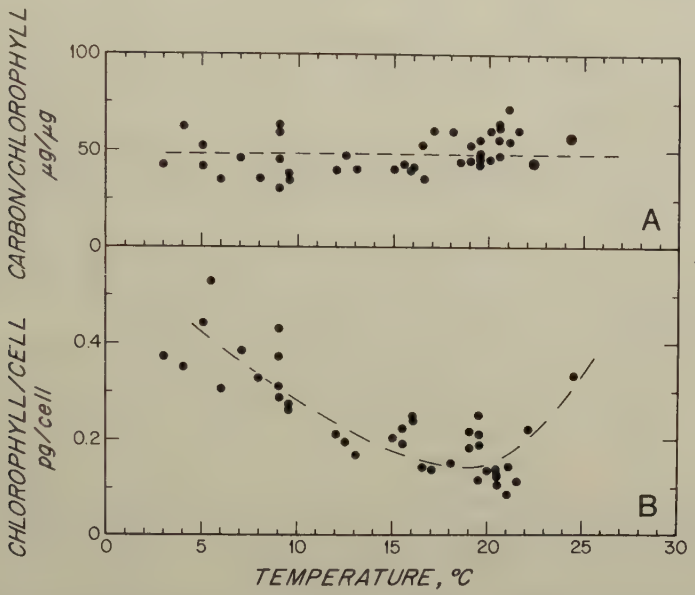


Fig. 3. Temperature-dependent variations in carbon : chlorophyll ratio (A) and cellular chlorophyll for *P. tricornutum* grown in outdoor continuous cultures (B).

TABLE I

Variations in cellular chemical constituents during winter bloom of *Skeletonema costatum* in outdoor mass cultures.

| Variable | Average for period | |
|---|---|---|
| | Dec. 1976 | Feb. 1977 |
| Pond temperature, °C | 0 | 0 → 5 |
| Solar irradiation, ly · day $^{-1}$ | 145 | 210 |
| pg carbon · cell $^{-1}$ | 30 | 31 |
| pg nitrogen · cell $^{-1}$ | 4.9 | 5.1 |
| Carbon : nitrogen ratio, µg : µg | 6.1 : 1 | 6.1 : 1 |
| pg chlorophyll *a* · cell $^{-1}$ | 299 | 289 |
| Carbon : chlorophyll ratio, µg : µg | 100 : 1 | 107 : 1 |
| Nitrogen : chlorophyll ratio, µg : µg | 16 : 1 | 18 : 1 |

### CELLULAR CHLOROPHYLL CHANGES

Seasonal changes in cellular chlorophyll in *Phaeodactylum tricornutum* followed those for carbon and nitrogen very closely (Fig. 3B). As a result, the carbon : chlorophyll ratio ($\mu g : \mu g$) remained constant at $\approx 50 : 1$ regardless of pond temperature (Fig. 3A). For *Skeletonema costatum*, on the other hand, virtually no changes were discernable in the various cellular constituents over a 5 °C range, but the carbon : chlorophyll ratio was $\approx 100 : 1$ (Table I).

## Discussion

### SPECIES DOMINANCE

The control and manipulation of phytoplankton species in large-scale outdoor cultures is perhaps the major unresolved problem in mass cultivation technology (Goldman, 1979a). The prohibitive cost of sterilizing very large quantities of medium for large-scale outdoor cultivation is compounded by problems of subsequent contamination with ubiquitous weed species which are ever present and which rapidly displace desired species in open outdoor systems.

In the current experiments we once again were able to show the importance of temperature in affecting seasonal changes in phytoplankton speciation. The species change in the waste-water-fed pond from *Phaeodactylum tricornutum* at temperatures >10 °C to *Skeletonema costatum* as the temperature dropped below 10 °C and then back to *Phaeodactylum tricornutum* when the temperature again rose above 10 °C (Fig. 1) is identical to results from previous years (Goldman & Ryther, 1976). It is now clear, however, that such changes will not occur when one of the species (that would normally compete favorably in a certain temperature range) is deprived of a particular nutrient that its competitor does not require. By simply replacing the mixture of waste water and sea water with sea water enriched with only N and P, *P. tricornutum* remained the dominant species over a temperature range of 0–25 °C. We did not discern what difference between the two media led to this altering of species dominance. The relative abundance of silicon in domestic waste water (Dunstan, 1975), however, and its absence in the required proportions with N and P in the enriched sea-water medium is a prime candidate cause for this difference. *P. tricornutum* is a unique diatom that does not have a silicon requirement (Lewin *et al.*, 1958; D'Elia *et al.*, 1979), whereas *Skeletonema costatum* has a characteristic demand for this nutrient which is common to diatoms in general (Werner, 1977).

### PHYTOPLANKTON CHEMICAL COMPOSITION

U-shaped variations in the cellular constituents of *Phaeodactylum tricornutum* with temperature (Fig. 2B, C) are virtually identical to previous results with this and

other species (Jorgensen, 1968; Goldman & Ryther, 1976; Goldman, 1977a, b). Williams (1971) and Goldman (1977b) have explained this phenomenon as a temperature-dependent uncoupling between growth and nutrient uptake, which is manifested as a change in cell size.

It is now well established that phytoplankton cell size is influenced by the degree and type of nutrient limitation as well as by temperature. For example, it has been shown repeatedly that at constant temperature the cell quota ($Q$ = cellular limiting nutrient concentration) varies from a minimum value ($k_Q$) at zero growth rate to a maximum ($Q_m$) at the maximum growth rate ($\hat{\mu}$) for numerous limiting nutrients (Fig. 4C) (Droop, 1974; Goldman & McCarthy, 1978). What the combined effects are of type and degree of nutrient limitation and temperature has yet to be identified completely (Goldman, 1979b). Williams (1971) suggested that minimum cell size for a given species should occur at the optimum temperature for growth (Fig. 4A, B). In addition, when nutrients are saturating and light is constant $\hat{\mu}$ can be

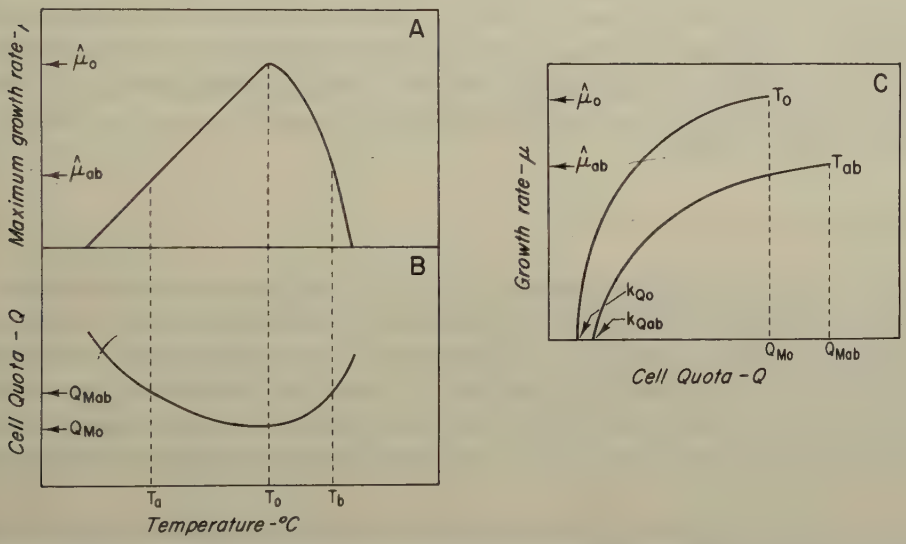


Fig. 4. Effect of temperature on phytoplankton growth and cellular constituency: A, maximum growth rate versus temperature; B, cell quota versus temperature; C, temperature-dependent relationships between nutrient-limited cell quota and growth rate.

described solely as a function of temperature (Eppley, 1972; Goldman & Carpenter, 1974). For these conditions $Q$ for different cellular constituents appears to be constant, independent of growth rate, and is equal to the respective $Q_m$ (Panikov & Pirt, 1978; Goldman, 1976b, 1979b).

In the current experiments nutrient saturation appeared to be sustained throughout the study period, as evidenced by the constant and low carbon : nitrogen ratio of $\approx 5.8 : 1$ (Fig. 2A) (D'Elia *et al.*, 1977; Goldman *et al.*, 1979), even though ir-

radiance levels varied 4- to 5-fold (Fig. 1A). The relative growth rate of the outdoor cultures (percentage of $\hat{\mu}$ – which in this study was temperature dependent because the dilution rate was maintained at 0.12–0.25 · day$^{-1}$) varied from a very high rate at low temperatures during winter to a low rate in the spring. In the light of the above conditions, the data for the carbon (Fig. 2B), nitrogen (Fig. 2C), and chlorophyll (Fig. 3B) cellular contents represent the respective maximum values at a particular temperature, and thus reflect a true temperature effect that is independent of growth rate.

When a nutrient such as nitrogen is limiting, distinct relationships between $Q$ and $\mu$ will exist (Fig. 4C) for different temperatures within a range in which an organism is capable of growing (Fig. 4A). The actual shape of the curves in Fig. 4C will be influenced strongly by temperature. For the optimum temperature ($T_0$) the relative $Q_m$ will be a minimum ($Q_{mo}$ in Fig. 4B) and $\hat{\mu}$ a maximum ($\hat{\mu}_0$ in Fig. 4A); but at $T_a$ and $T_b$ on either side of the optimum temperature (for convenience $Q_{ma} = Q_{mb}$ and $\hat{\mu}_a = \hat{\mu}_b$) $Q_{mab}$ (Fig. 4B) and $\hat{\mu}_{ab}$ (Fig. 4A) will be respectively greater and lower than at $T_0$. Thus, when there is a deviation in temperature from the optimum the net effect will be to flatten out and lower the asymptote of the $Q$ vs. $\mu$ curves. Just how $k_Q$ will be influenced by temperature has not been identified clearly; but some evidence points to a similar temperature-dependency of this parameter (Ahlgren, 1978; Goldman, 1979b).

## CELLULAR CHEMICAL RATIOS

We could not discern any significant effect of temperature on both the carbon : nitrogen and carbon : chlorophyll ratios in *Phaeodactylum tricornutum* between 3–25 °C (Figs. 2A and 3A) and *Skeletonema costatum* between 0–5 °C (Table I). In both cases the carbon : nitrogen ratio by weight was sustained at ≈6 : 1. As mentioned earlier, such conditions reflect nutrient saturation; and, in fact, these ratios are within the range (5 : 1–6 : 1) of minimum levels possible with phytoplankton under ideal conditions.

The carbon : chlorophyll ratio in phytoplankton, like $Q$, appears to be affected by both degree of nutrient limitation and by temperature (Eppley, 1972). For *Phaeodactylum tricornutum* the constant and low carbon : chlorophyll ratio of 50 : 1 (Fig. 3A) is consistent with the carbon : nitrogen data (Fig. 2A) in indicating nutrient saturation. The cellular chlorophyll content vs. temperature curve (Fig. 3B), however, has the same U-shape as the cellular carbon and nitrogen curves, demonstrating a very tight coupling between carbon, nitrogen, and chlorophyll synthesis over the temperature range 3–25 °C. As with cellular carbon and nitrogen, the cellular chlorophyll was a minimum at the optimum temperature for growth (Fig. 3B).

For *Skeletonema costatum*, in contrast, because the carbon : nitrogen ratio of 6 : 1 between 0–5 °C clearly represents nutrient saturation, a relatively high carbon : chlorophyll ratio of 100 : 1 is suggestive of a temperature-dependent uncoupling between

chlorophyll and carbon synthesis. Unfortunately, because of the narrow temperature range in which *S. costatum* dominated, it was impossible to discern if and how temperature influences this parameter. Intuitively, a carbon : chlorophyll ratio of $\leqslant 50:1$ should coincide with a carbon : nitrogen ratio of $\approx 6:1$ (Eppley & Renger, 1974; Perry, 1976; Harrison *et al.*, 1977); but in the case of *S. costatum* coupling between chlorophyll and nutrient synthesis may occur only close to the optimum temperature for growth which is $\geqslant 5\,^{\circ}C$ (Smayda, 1973). For example, the very high cellular carbon content in *S. costatum* between 0–5 °C ($\approx 30$ pg $C \cdot cell^{-1}$) found in this study, is consistent with the increasing values (10 to 20 pg $C \cdot cell^{-1}$) found by Jorgensen (1968) for the same species at decreasing temperatures in the range $20 \rightarrow 7\,^{\circ}C$. Similar variations in the carbon : chlorophyll ratio of *S. costatum* were found for conditions of constant temperature, but varying degrees of nitrogen limitation; from 61 under nutrient saturation at 18 °C to 98 when ammonium starvation existed (with a corresponding increase in the carbon : nitrogen ratio from 5 : 1 to 14.8 : 1) (Harrison *et al.*, 1977), and from 26 : 1 during the first days of nitrate-limited growth at $\approx 20\,^{\circ}C$ to $>100:1$ as nitrogen limitation became increasingly more severe (Steele & Baird, 1962). Thus, it appears that *Skeletonema costatum*, unlike *Phaeodactylum tricornutum*, displays a temperature-dependent uncoupling between carbon and chlorophyll synthesis that is not associated with degree of nutrient limitation.

## CONCLUSIONS

Temperature clearly plays an important role in influencing the outcome of competition among phytoplankton species in enriched cultures. The outcome of competition can be shifted, however, by simply eliminating an essential nutrient such as silicon that is required only by certain species. Recently, Turpin & Harrison (1979) showed that a similar displacement of species could be achieved by providing a particular essential nutrient (e.g. nitrogen) in a pulsed rather than a continuous mode.

Both nutrient limitation and temperature have a dramatic effect on the size of cells and the resulting cellular chemical composition. To examine the role of temperature on phytoplankton dynamics it is essential that experiments be designed so that nutrients are non-limiting. Thus, cells must be grown at their maximum rate under nutrient saturation in batch cultures, or under nutrient saturation at sub-maximal growth rates in continuous cultures as in the present study, or finally, at dilution rates close to $\hat{\mu}$ (Goldman & Peavey, 1979).

The U-shaped response in cellular constituency of phytoplankton to temperature seems to be a common feature in many species, although the degree of change both in magnitude and effect on particular constituents is species specific. How important these types of responses are in influencing the outcome of competition is still unanswered. For a given species, however, minimum cell size and cellular con-

stituency appear to occur at the optimum temperature for growth, as depicted in Fig. 4.

## Acknowledgements

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# UPPER TEMPERATURE TOLERANCE AND THE EFFECTS OF TEMPERATURE ON BYSSUS ATTACHMENT IN THE QUEEN SCALLOP, *CHLAMYS OPERCULARIS* (L.)

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**Abstract:** The upper temperature tolerances ($LD_{50}$–48 h) have been established for three size groups of *Chlamys opercularis* (L.) at different acclimation temperatures. In large and intermediate-sized scallops mortality was between 19 and 24 °C, and in spat between 24 and 25 °C. These mortalities varied according to the thermal history of the animals.

The rate of byssus formation in spat was found to be maximum at 18 °C.

## INTRODUCTION

The work described in this paper formed part of a programme to establish the biological feasibility of cultivation of the queen scallop, *Chlamys opercularis* (Brand *et al.*, 1980) and it sought to establish some of the effects of temperature and temperature change with a view to optimizing temperature conditions for metabolism, growth, and survival. Fry (1947), Kinne (1970), and Newell (1970) have reviewed the large amount of literature concerning the effects of temperature on aquatic organisms but there have been few such studies on pectinid bivalves. Dickie (1958) and Dickie & Medcof (1963) studied the survival of *Placopecten magellanicus* at high temperatures. Sastry (1961) established the upper lethal temperature of *Aequipecten irradians concentricus*, while Nakanishi (1977) made temperature studies on *Patinopecten yessoensis* and McLusky (1973) investigated the effects of temperature acclimation on the filtration rate and oxygen consumption of *Chlamys opercularis*. It is well known that tolerance to high temperatures and temperature change can be modified by the previous thermal history of an organism; thus thermal acclimation, or the level to which an animal is physiologically adjusted, must be taken into account in any such study.

Widdows (1973) defined three levels of metabolism in *Mytilus edulis*: "standard" – the minimum energy requirements, "routine" – that which includes the energy demands of spontaneous activity, and "active" – oxygen consumption under maximum activity. Byssus attachment in bivalves can probably be considered as routine metabolism, albeit at a high level, and has been studied in pectinids by Caddy (1972) and Soemodihardjo (1974). Roberts (1973) proposed that byssogenesis could be a useful and rapid bioassay of potentially toxic substances, and

demonstrated that byssogenesis in *Chlamys opercularis* was extremely sensitive to sub-lethal concentrations of pesticide.

The aims of this study were, therefore, to investigate the lethal and sub-lethal effects of temperature change on *C. opercularis* (L.) by (a) establishing experimentally the limits of upper temperature tolerance and (b) assessing the rate of byssus attachment of spat as an indication of metabolism and activity at different temperatures.

## Materials and Methods

### Upper lethal temperature

Three size groups of *C. opercularis* were used for the temperature tolerance experiments. Animals in Group A (large, <60 mm shell height) and Group B (intermediate, 30–40 mm shell height) were obtained by dredging in an area 2 miles southwest of Chicken Rock, Isle of Man, and those in Group C (spat, 5–10 mm shell height) were obtained from artificial collectors (Brand *et al.*, 1980). Experiments on Groups A and B were carried out between April and October 1976 and 1977, and Group C in September–October 1977. The ambient sea temperature off Port Erin increased from ≈7 °C to 14 °C between April and October.

Animals were maintained in flowing sea water and their temperature gradually adjusted to four acclimation temperatures (5, 10, 15, and 20 °C). At least 1 day/°C temperature change was allowed for acclimation, and usually the animals spent 3 to 4 wk at the final temperatures before being used in experiments. The apparatus consisted of 21-polythene containers suspended in temperature controlled water baths. Each container was provided with gentle aeration and a subsurface inflow of fresh sea water at a rate of ≈500 ml/h.

The upper lethal temperature was expressed as that temperature required to kill 50% of the experimental population in a 48-h test exposure ($LD_{50}$–48 h). The experimental populations were transferred directly from the acclimation temperature to the test temperature. Any dead animals were removed during the experiment and those surviving after 48 h were returned to tanks with flowing sea water at their original acclimation temperature and mortality subsequently recorded. Twenty animals were normally used for each individual experiment, with three to four animals per container in Groups A and B, whereas all those in Group C were placed in a single container.

Further experiments were also carried out to remove the effects of acclimation and temperature shock. In these, animals were placed in test containers at their natural acclimation temperature (ambient) and the temperature raised at a rate of 1 °C/day until death of 50% of the experimental population occurred.

Dickie (1958) used velar contractility as the criterion for death of *Placopecten*, although this may be an excessively severe method since a state from which the

animal will not recover may be reached long before the velar contraction response is lost. This criterion was nevertheless used in this study since it is a relatively consistent and easily detected response. Overall mortalities were not recorded, however, until after a 2-day recovery period, thus including those animals which showed the velar contraction response at the end of the test period but which were too severely damaged to recover.

### BYSSUS ATTACHMENT

Experiments on byssus attachment were only carried out on spat because, in pectinids, the tendency to produce byssus diminishes with increasing size (Caddy, 1972; Soemodihardjo, 1974). *Chlamys opercularis* spat were maintained in flowing sea water for at least 1 wk before use (ambient temperature 12–14 °C). They were transferred to the experimental containers at ambient temperature and the temperature was gradually adjusted to the required level over a 12-h period; a further 24 h was then allowed before the experiments began. The apparatus was as described previously, but with spat placed individually in the 2 × 2 × 2 cm compartments of plastic compartmented dishes. These dishes were used to prevent interaction between individuals as well as provide vertical surfaces on which *C. opercularis* spat, in common with spat of other pectinids (Sastry, 1965; Castagna, 1975) readily attach.

The experiments were started by detaching the animal from its anchorage by cutting the existing byssus with a scalpel. At certain time intervals (initially 5 min) the number of spat which had re-attached were recorded. Attachment was detected by applying a gentle water jet to the animal from a pipette, taking care to avoid undue disturbance. Twenty animals were used in each test, and at least six replicate experiments were carried out at various temperatures from 6 to 24 °C. Experiments were terminated after 300 min. Illumination was from a 60-W fluorescent strip light 1 m above the water baths and each container was covered with an opaque polythene lid.

## RESULTS

### UPPER LETHAL TEMPERATURES

The comparative temperature–mortality curves (Fig. 1) for the different size groups show the typical sigmoid pattern expected from this type of experiment (Kinne, 1970) with a relatively small difference in test temperature causing a change from 0–100% mortality. This was particularly so for the spat (Group C) where only 1–2 °C separate the extremes of mortality; in Groups A and B a 2–4 °C change was more usual. Similarly, the mortality curves for Groups A and B at different acclimation temperatures showed a spacing of about 2 °C against the *x*-axis but the curves for Group C were bunched at rather higher temperatures, with only about 2 °C separating all four curves.

Values for median lethal temperatures ($LD_{50}$–48 h) were derived from these curves and are plotted against acclimation temperature in Fig. 2. The slopes and elevations of the resulting lines were very similar for Groups A and B and it appears

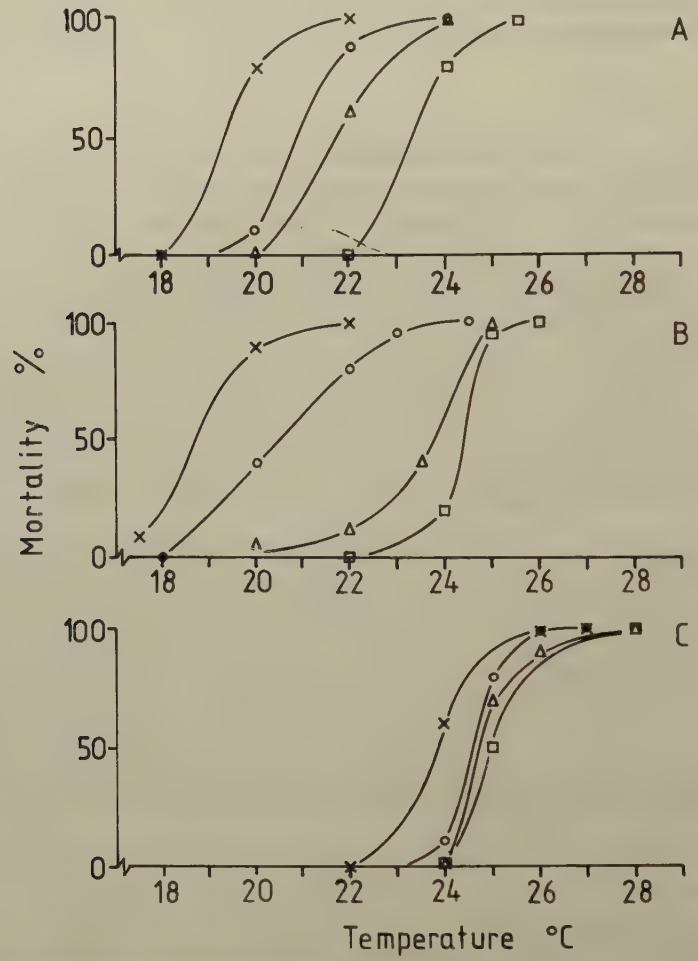


Fig. 1. Temperature–mortality curves for the three size groups (A, <60 mm; B, 30–40 mm; C, 5–10 mm shell height) of *Chlamys operecularis* from four acclimation temperatures: ×, 5 °C; ○, 10 °C; △, 15 °C; □, 20 °C.

that a 5 °C change in acclimation temperature resulted in a change in the median lethal temperature of ≈1.25–2.50 °C. In Group C, however, the line was elevated, the slope shallower and the corresponding change in median lethal temperature was only ≈0.6 °C.

The temperature at the intersection between the lines for median lethal tempera-

ture and the construction line (i.e., where acclimation temperature = median lethal temperature) gives an approximation of the maximum upper lethal temperature, when no increase in $LD_{50}$–48 h results from a further increase in acclimation temperature. Rough linear extrapolations of the existing data give approximate values

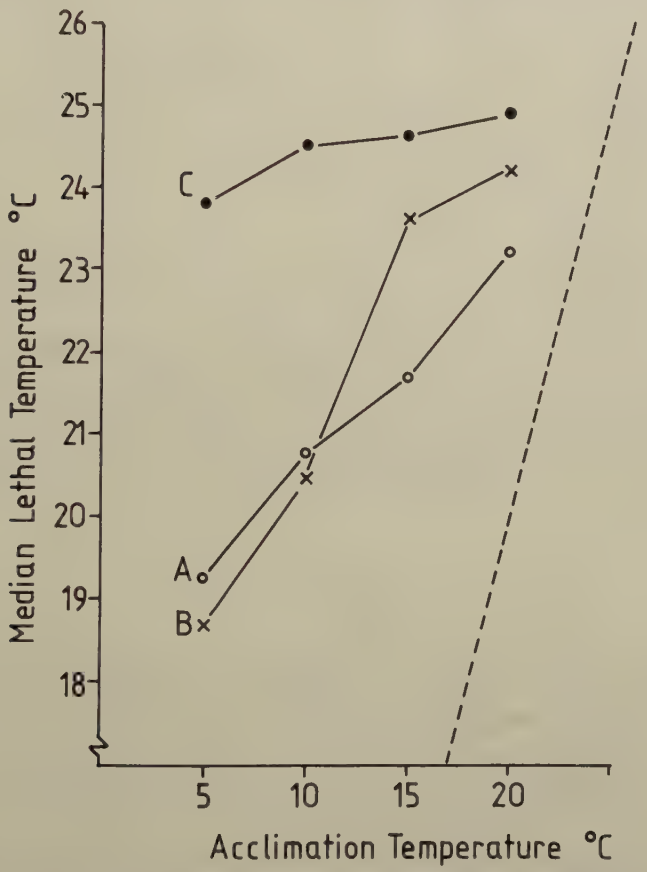


Fig. 2. Median lethal temperatures ($LD_5$–48 h) for the three size groups (A, <60 mm; B, 30–40 mm; C, 5–10 mm shell height) of *Chlamys opercularis* acclimated to different temperatures: the dashed line shows where acclimation temperature = median lethal temperature.

of 24 °C (Group A), 24.5 °C (Group B), and 25 °C (Group C). The slow heating "continuous acclimation" experiments may give a more realistic indication of the maximum upper lethal temperature. In all cases, death occurred over the very small temperature change of 1–2 °C and the temperatures at which 50% of the experimental population had died were 26.5 °C (Groups A and B) and 27.5 °C (Group C); about 2 °C higher than the values estimated from Fig. 2, with a very small difference between the size groups.

BYSSUS ATTACHMENT

When the percentage of spat that had become attached was plotted against time, most of the resulting graphs were sharply curvilinear, as found by Caddy (1972) for *Placopecten* and by Soemodihardjo (1974) for *Chlamys opercularis*. Representative results for single experiments at 9, 15, and 21 °C are shown in Fig. 3. The initial rapid

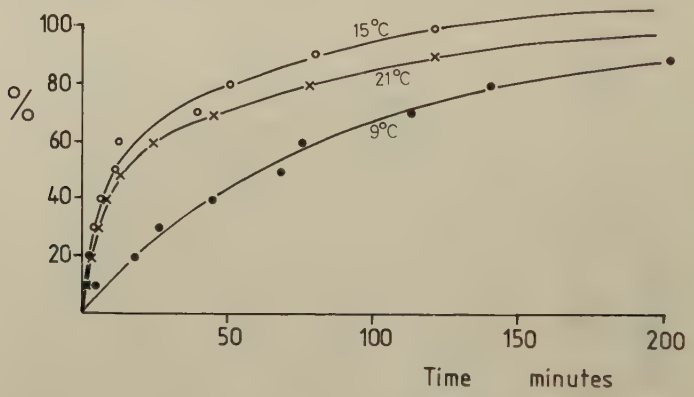


Fig. 3. Percentage of *Chlamys opercularis* which had byssally attached during the experimental time period at three different temperatures.

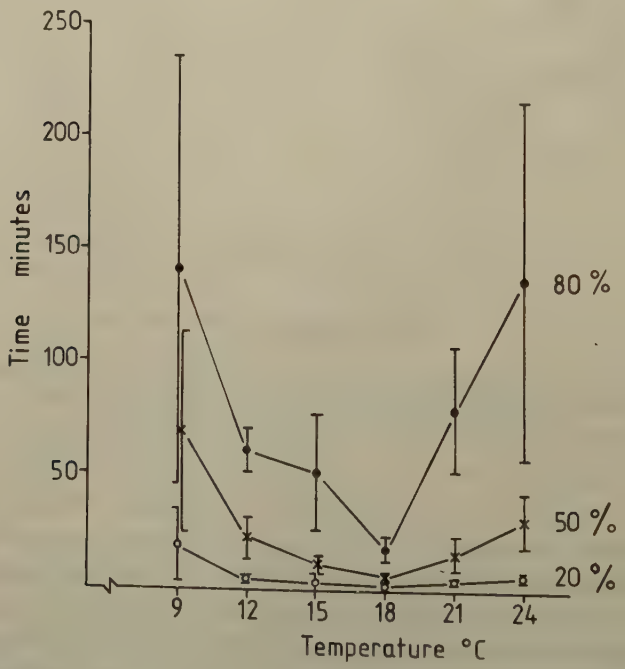


Fig. 4. Time taken to reach 20, 50, and 80% byssal attachment at different temperatures: mean values with 95% confidence limits.

increase in percentage attached gradually fell as the experiments progressed and an equilibrium between numbers attaching and detaching may eventually be reached, as found by Caddy (1972).

To allow comparisons between the different temperatures, three arbitrary levels of attachment were chosen: at 20, 50, and 80%. The time taken to reach these levels of attachment was estimated for each experiment and the mean values at each temperature shown in Fig. 4. The rate of byssal attachment at 6 °C was extremely low, with usually only 10–20% attachment reached in 300 min, so these data have not been included. Fig. 4. shows that at the two extremes of temperature (9 and 24 °C), the rate of byssus attachment was slow and highly variable. Towards these temperature extremes, the animals showed little activity and the drop in the numbers attaching did not appear to be caused by any increase in activity, such as swimming. Such increases in activity or escape responses to adverse conditions would be antagonistic to byssus attachment but may only occur under conditions of abrupt temperature change, when no acclimation is possible. Both the time and variability of attachment rate diminished towards the mid-range of temperature to give an "optimum", or maximum rate of byssus attachment at 18 °C. These temperature differences were more apparent in the 80% level than in the 50 and 20% levels, respectively, indicating that the maximum possible attachment level should be used to test for environmental or chemical sensitivity.

## Discussion

The results illustrate that both acclimation temperature and size can have a modifying effect on temperature tolerance. The data obtained here can, however, only be related to a 48-h exposure period for, as shown by Dickie (1958) and Dickie & Medcof (1963), a longer exposure period would result in lower lethal temperatures while a shorter exposure period would allow higher temperatures to be tolerated. Kennedy & Mihursky (1971) studied the temperature tolerance of various estuarine species of bivalve and Dickie (1958) found that the upper lethal temperature of *Placopecten* varied from 20–24 °C depending on acclimation temperature. *Chlamys opercularis* gave similar results with mortality occurring in intermediate and large scallops between 19 and 24 °C and in spat between 24 and 25 °C. The occurrence of these mortalities is dependent on the thermal history of the individuals and the duration of exposure.

Brett (1956) emphasized that age and size have a modifying effect on lethal temperature, although McLeese (1956) found no such evidence. Huntsman & Sparks (1924), however, stated that resistance to heat diminishes with increasing size and, although Dickie (1958) showed no consistent differences in the thermal tolerances of large and small *Placopecten*, he later indicated that very young scallops were more resistant to high temperatures (Dickie & Medcof, 1963). From Fig. 2, it ap-

pears that spat of *Chlamys opercularis* (Group C) are more tolerant of high temperatures, and the more horizontal line also indicates that the effects of acclimation are reduced. The close similarity in all the values obtained, however, for the maximum upper lethal temperature suggests that the differences between the three size groups may have arisen from differences in resistance to thermal shock rather than inherent differences in the upper temperature tolerances.

Sastry (1961) found the upper lethal temperature of *Aequipecten* to be between 34.5 and 35.2 °C but he used a method involving rapid heating similar to that used by Henderson (1929) who established the upper lethal temperature of a variety of species by raising the temperature at a rate of 1 °C/5 min. Similar experiments in this study showed that *Chlamys opercularis* could be heated at a rate of 1 °C/10 min to a temperature as high as 28 °C with no mortality after return to normal temperatures and this result shows that care must be taken when interpreting results of experiments using this type of procedure. Under rapid heating, the animals gaped and became quiescent, not reacting to touch, but recovery was rapid on return to lower temperatures. These responses to thermal shock when no acclimation is possible due to the abruptness of the temperature change, were similar to those described by Dickie (1958) for *Placopecten*, which also produced large quantities of mucus. Dickie & Medcof (1963) suggested that a similar situation may exist in scallops to that postulated for the lobster by McLeese & Wilder (1958) who described thermal zones of activity and inactivity, both within a zone of thermal tolerance, where temperature changes could induce debility without actually killing the animals. Furthermore, they postulated that such debilitating temperature changes may indirectly affect survival by inhibiting normal escape reactions.

The "optimum" temperature for byssus attachment of 18 °C agrees well with results given by McLusky (1973), who found that the oxygen consumption and filtration rates of acclimated *Chlamys opercularis* were at a maximum at 15 °C. He also found that oxygen consumption fell sharply at 20 °C and suggested that this was near the lethal temperature. Caddy (1972) found an increase in the number of byssus threads produced by *Placopecten* between 2.5 and 15 °C, but did not experiment above this temperature range. Glaus (1968) found that thread production in *Mytilus edulis* increased over a temperature range of 18 to 28 °C but Van Winkle (1970) found that byssus formation approached zero and *M. edulis* died at temperatures exceeding 26 °C.

Fry (1947) called the energy available for external work the "scope for activity" and this is determined from the difference between active (maximum) and standard (minimum) rates of metabolism. Bayne *et al.* (1973) further distinguished between the "maximum scope for activity" (active minus standard) and the more ecologically significant "routine scope for activity" (routine minus standard). Many workers have found that temperature affects the active rate of metabolism more than the standard rate and that the two curves for these measurements converge at temperature extremes (Halcrow & Boyd, 1967; Newell, 1970; Newell & Pye, 1970a, b; Wid-

dows, 1973). The last author found that the "maximum scope for activity" and filtration rate in *M. edulis* under acute temperature change, were greatest at 20 °C. Ali (1970) also found that the maximum filtration rate of *Hiatella arctica* was between 15–17 °C and suggested that the optimum temperature range for boreo-arctic species is 15–18 °C. The peaks of oxygen consumption and filtration rate of *Chlamys opercularis* at 15 °C (McLusky, 1973) and the 18 °C optimum for rate of byssus attachment agree well with the above observations. It would be useful, however, to establish the relationship between the effect of temperature on byssus attachment and on other rates, such as respiration, pumping and feeding, under the same conditions. Nevertheless, the results suggest that byssus attachment may be a useful and readily observed technique for establishing temperature optima and may indicate the temperature range at which the "routine scope for activity" in *C. opercularis* is highest under the conditions studied.

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# THE REGULATION OF CALCIUM AND MAGNESIUM IN THE URINE DURING THE MOULTING CYCLE OF *CRANGON VULGARIS* (Fabr.)

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**Abstract:** *Crangon vulgaris* (Fabr.) in brackish water (*S*, 10‰; temperature, 10 °C) was found to have a urine : haemolymph ratio for magnesium ranging from 1.5 : 1 to 5 : 1. Haemolymph magnesium concentration throughout the moulting cycle was held far below the outer medium, while the urine magnesium was higher than in the medium. Calcium concentration in both haemolymph and urine was always above the medium concentration. The urine : haemolymph ratio for calcium was $<1:1$.

## INTRODUCTION

The function of the antennal gland of brackish water decapod crustaceans and the composition of the urine has been little studied. Special problems of salt and water balance arise for brackish water animals living in varying salinities and thus in a varying ionic composition. A conservation of certain ions by re-absorption in the urine could here be considered an advantage. In most cases the urine, even for osmoregulating species, has been found to be isosmotic to the blood (see Smith, 1976, for review). The urine production rate and the physiology of the antennal gland of *Carcinus maenas* have been thoroughly studied by Binns (1969a, b). Studies on the urinary composition of brackish water Natantia are confined to those on *Palaemon serratus* and *Palaemonetes varians* by Parry (1954, 1955), *Palaemon macrodactylus* by Born (1968), *Macrobrachium australiense* and *M. equidens* by Denne (1968), and *Crangon vulgaris* by Spaargaren (1971), Hagerman & Larsen (1977) and Hagerman (1978). The two *Macrobrachium* species had a hypo-osmotic urine, all the rest had an isosmotic urine. The urine : blood ratio for chloride in intermoult *Crangon vulgaris* was 1 : 1, while sodium was slightly more, 1.2 : 1 (Hagerman, 1978). A higher sodium concentration in the urine compared with the blood was also found for *Pachygrapsus crassipes* by Prosser *et al.* (1955) in 50% sea water ($\approx 17$‰ *S*). Since the urine in *Crangon* is isosmotic, to achieve electrochemical balance in the urine the balance must be created by another cation being retarded or re-absorbed in the excretory system. This problem is accentuated as ammonium ions are also excreted via the urine, especially in low salinities, thus adding to the positively charged ions (Mangum *et al.*, 1976).

Re-absorption of ions is said to take place in the nephridial canal between labyrinth and bladder (Peters, 1935) but *Crangon* lacks the nephridial canal (Parry, 1960) and

thus an eventual re-absorption of ions and water, i.e. the difference between primary and final urine must take place in the labyrinth and bladder.

The aim of the present paper is to investigate the regulation of calcium and magnesium in the urine. Since calcium in the blood shows a pronounced variation with the moulting cycle (Hagerman, 1973) the concentration of ions in the urine in relation to the moulting cycle of the species has been investigated. The flow rate and volume of the urine of *C. vulgaris* as well its osmotic concentration and sodium and chloride regulation has already been described (Hagerman & Larsen, 1977; Hagerman, 1978).

## Material and Methods

*C. vulgaris* (Fabr.) was collected in shallow water (depth, 1 m; *S*, 10–20‰; temperature, 15 °C; fully oxygenated) off Julebaek in the Øresund north of Helsingør, Denmark, in summer. In the laboratory the animals were placed singly in small containers. All animals used for the experiments had spent at least 1 wk under the experimental conditions (*S*, 10‰; temperature 10 °C) prior to study. The animals were fed with *Mytilus* flesh and mysids. Moulting stages were noted and classified according to Drach (see Passano, 1960).

Blood and urine samples were taken following the technique of Hagerman (1978). Haemolymph and urine samples were diluted 1500 times in 0.75% EDTA-$Na_2$ and analysed in a Unicam SP 90 Series 2 atomic absorption spectrophotometer and recorded on a Goerz Servogor RE 511 recorder.

## Results

The magnesium content of the haemolymph is far below that of the outer medium (13.5 mM/l) and throughout the moulting cycle lies at around 5–10 mM/l with lowest values in the intermoult stages C–$D_0$ (Fig. 1). It has been suggested that the increase in magnesium content close to ecdysis is caused by an increased water uptake and consequent increases in the ion fluxes (Hagerman, 1973).

The urine magnesium during the moulting cycle is also shown in Fig. 1 and is always far above that of the haemolymph and the medium. There is a tendency towards a higher excretion of magnesium immediately after ecdysis, a minimum in the intermoult period and again an increase in the pre-ecdysis stage. These changes are not surprising as the animal must excrete as much magnesium as possible in the pre- and post-moult stages in a vain attempt to keep haemolymph magnesium concentration as low as in the intermoult stages.

The urine : blood (U : B) ratio for magnesium varies from $\approx 1.5:1$ to $>5:1$. When plotted (Fig. 2) the U : B ratios for magnesium apparently fall into two groups, 1.5–2.5 : 1 and 3.5–4.5 : 1. Considering the sampling technique and the

processes involved in urine production it is possible that the low ratio (1.5–2.5 : 1) represents a urine not finally modified and the high U : B ratio (3.5–4.5 : 1) represents the final urine. The stimulation of the nephridiopore with a hair or small needle prior to sampling can force the animal to urinate and, if the bladder contains none or only a small volume of final urine, it will be mixed with urine not ready for excretion, thus decreasing the level of urinary magnesium. The site of magnesium secretion has already been shown to be the bladder wall in *Pachygrapsus crassipes* and *Carcinus maenas* by Gross & Capen (1966) and Lockwood & Riegel (1969).

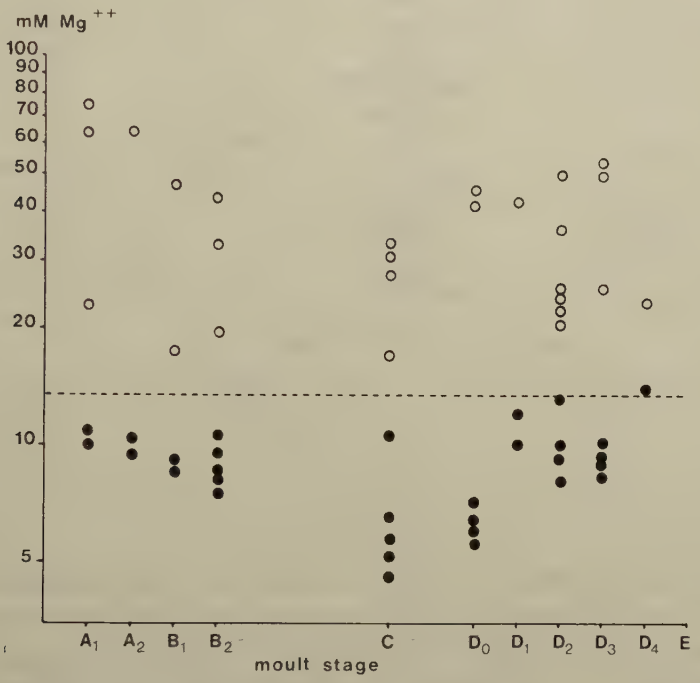


Fig. 1. The regulation of magnesium in haemolymph and urine during the moulting cycle of *Crangon vulgaris* in a salinity of 10‰ at 10 °C: ○, urine magnesium; ●, haemolymph magnesium; --, medium concentration.

Thus it is suggested that the U : B ratio will be low in the urine initially discharged into the bladder and the ratio will then increase as magnesium secretion from the bladder wall occurs, until the final urine expelled from the animal has a higher U : B ratio.

The U : B ratio for calcium is shown in Fig. 3. Severe difficulties in analysing urine calcium meant that no samples in late pre-ecdysis and early post-ecdysis could be taken and, therefore, Fig. 3 only shows a U : B ratio below unity in the intermoult stages C–$D_2$. Haemolymph calcium showed the same pattern as found by

Hagerman (1973), i.e. lowest immediately after ecdysis, increasing in stage B and early C, then a decrease and a subsequent mobilization of the stored calcium very close to ecdysis, being at levels above the medium concentration throughout

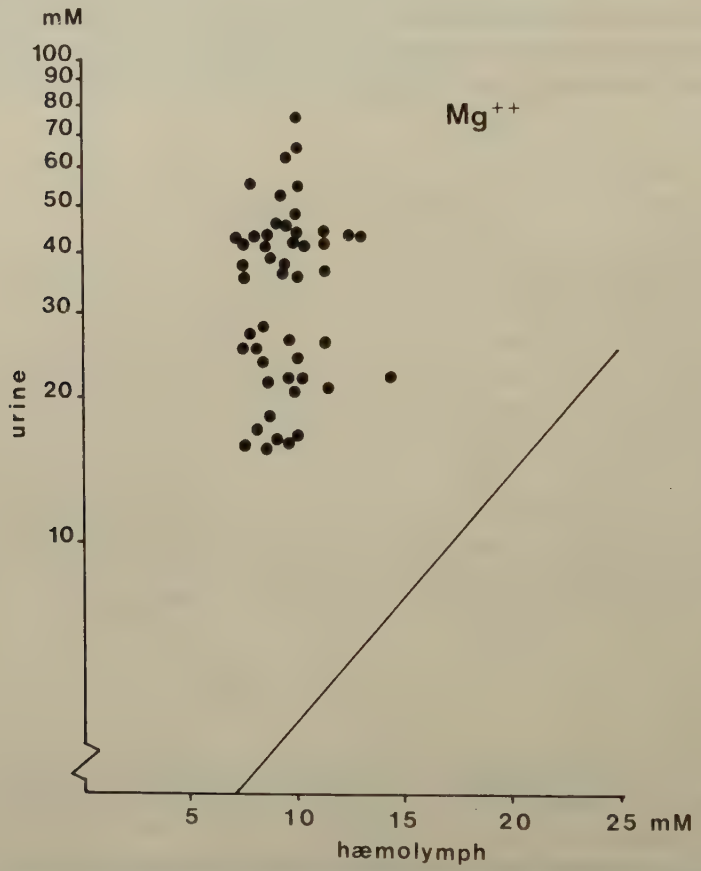


Fig. 2. The U : B ratio for magnesium in *Crangon vulgaris* in a salinity of 10‰ at 10 °C: the line indicates U : B = 1.

the moulting cycle. In the experimental salinity of ≈10‰ the haemolymph concentration of calcium in the intermoult stages was 7.5–10 mM/l, the corresponding urine concentration around 5 mM/l and the medium was 2.7 mM/l. A lower blood and urine concentration is seen in a few cases in Fig. 3, these are from animals held in a salinity <10‰. In all cases blood and urine calcium was held above the medium concentration.

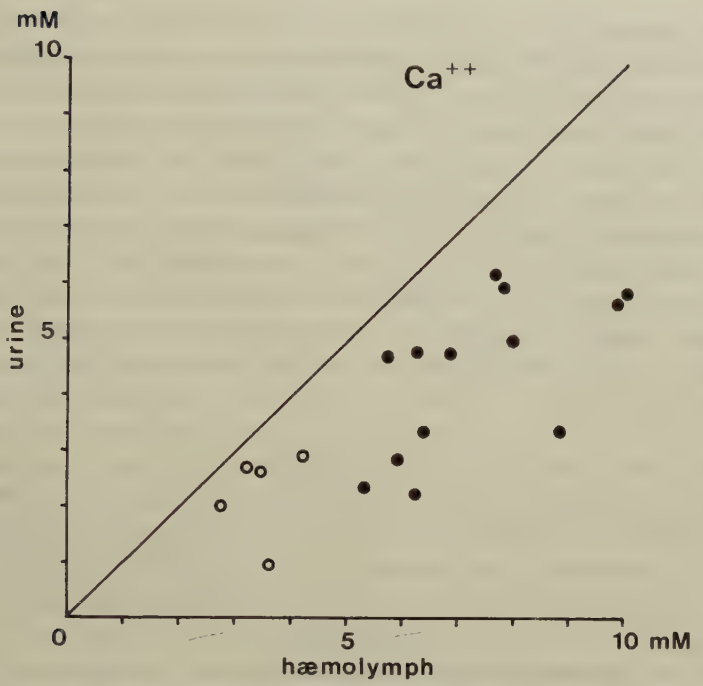


Fig. 3. The U : B ratio for calcium in *Crangon vulgaris* in a salinity of 10‰ at 10 °C (○ at <10‰): the line indicates U : B = 1.

## Discussion

That *Crangon vulgaris* has an urine isosmotic to the blood (U : B, 1) has been found by Spaargaren (1971) and Hagerman (1978) for individuals from marine and brackish areas, respectively. The urine remained isosmotic to the blood during the whole moulting cycle. The U : B ratio for chloride was 1 : 1, while sodium was 1.2 : 1 (Hagerman, 1978). As *Crangon* has been found to have a rather high blood protein level (<90 mg/ml, Djangmah, 1970) the positive U : B ratio for sodium might be caused by urine formation by ultrafiltration. Such a deviation above unity for sodium has been shown earlier by Prosser *et al.* (1955) for *Pachygrapsus* while the only case with a deviation above unity for chloride was found by Malley (1977) for *Panulirus argus*.

As *Crangon vulgaris* is hyperosmotic in salinities <25‰ osmotic inflow of water will cause the animal to swell unless counteracted by an increased excretion of water via the antennary glands, i.e. via the nephridiopores. *Crangon* excretes ≈20 $\mu l \cdot g^{-1} \cdot h^{-1}$ in *S*, 10‰ during the intermoult, increasing to 50–60 $\mu l \cdot g^{-1} \cdot h^{-1}$ immediately after moulting (Hagerman & Larsen, 1977). These changes are caused by changes in water fluxes. For an animal like the shore crab, *Carcinus maenas*, living

in 100% sea water a considerable withdrawal of water from the urine can take place (Riegel & Lockwood, 1961). Such a withdrawal seems to be an adaptation to littoral life as exposure to air resulted in an enhanced water resorption. For *Crangon* in brackish water a water resorption has no meaning; on the contrary, the osmotic processes makes it necessary to excrete more water. For brackish water animals like *Crangon* a loss of vital ions with the urine must be prevented, while on the other hand unwanted ions like $Mg^{2+}$ entering the body due to the concentration gradient to the medium must be excreted at an increased rate. The problems are more accentuated at ecdysis when permeability is increased. Thus, the antennal gland needs to regulate the different ions independently either actively or passively. Of the hitherto investigated ions in the urine of *Crangon* chloride is the only one that is regulated in such a way that it is maintained isoionic to the haemolymph (Hagerman, 1978). Sodium in the urine of animals from brackish waters is held slightly above the haemolymph sodium concentration. It has been claimed that the excretion of magnesium might interfere with the excretion of sodium, thus causing a higher sodium concentration in the urine (Prosser *et al.*, 1955). This was the case for *Pachygrapsus crassipes* when the U : B ratio for magnesium increased to >6. On the other hand, Lockwood & Riegel (1969) found no direct connection between magnesium and sodium excretion. In *Crangon vulgaris* the U : B ratio is up to 3 for the urine present in the bladder shortly after a urination and up to 6 in the final urine, i.e. the urine ready for excretion, probably after active secretion of magnesium in the urinary bladder, as resorption of water is unnecessary in a brackish water species. Gross & Capen (1966) showed that the urine magnesium fluctuated with time, another indication that the urine in the bladder has its final composition just prior to excretion.

In the final urine the U : B ratio for magnesium is highest immediately after ecdysis when the excretory system is required to get rid of the increased osmotic inflow of water and ions caused by a higher permeability. Glynn (1968) found that the urine magnesium level during the moulting cycle was five to ten times the haemolymph level and no significant fluctuations were observed when *Homarus vulgaris* from fully marine waters were investigated. A high magnesium ratio was also found by Franklin (1975) for *Palaemon serratus* where the ratio declined from 33 : 1 in a salinity of 35‰ to only 1.3 : 1 in a salinity of 8‰. A lower U : B ratio was also noted for *Carcinus* in low salinities by Lockwood & Riegel (1969) and for *Pachygrapsus* by Gross & Marshall (1960) but was still above unity. Sulphate has not been measured in *Crangon vulgaris* but it is reasonable to suppose that *Crangon*, like most other decapods (Nicol, 1967), concentrates sulphate to an extent similar to magnesium and would appear to help to maintain the electrical balance in this way.

There are few references in the literature to the calcium level in the urine of decapods. Large amounts of calcium are needed for the mineral component of the new shell, and this calcium is derived directly from the haemolymph. Travis (1955) showed that in *Panulirus argus* in sea water both blood and urine calcium were

above the medium, with the U : B ratio >1, even when a minimum occurred in post moult stage B. In a medium where there is enough calcium there is no reason to save calcium within the body by having a U : B ratio <1. Glynn (1968) showed, however, that during the moulting cycle of *Homarus vulgaris* there is a U : B ratio of <1 except in stage B where it increased to 1. Both these investigations were made in full strength sea water. In *Pachygrapsus crassipes* the U : B ratio was >1 in 100% sea water (Prosser *et al.*, 1955). In brackish water it must be an advantage to have a U : B ratio <1 as this will conserve calcium in the haemolymph, which seems to be the case for *Crangon vulgaris*, where the U : B ratio is <1 all the time. This low ratio will also help to counteract the higher U : B ratio for sodium, thus helping both to keep the electro-neutrality of the urine and the urine isosmotic to the blood.

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# THE RESPONSE OF MEIOFAUNA TO SEDIMENT DISTURBANCE[1]

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**Abstract:** The meiobenthos inhabiting an intertidal mud bar were disturbed by hand-turning the sediment of a 9-$m^2$ area with a shovel and monitoring the subsequent recolonization process.

The immediate impact of the disturbance on this community dominated by Nematoda (91%), Copepoda (4%) and Foraminifera (4%), was to remove more than 70% of the meiofauna. However, after only one tidal cycle, total numbers of nematodes, copepods, foraminiferans and other meiofauna taxa were at predisturbance and control (similar 9-$m^2$ site on the same flat) density values. Nematode species assemblages rapidly adapted to the disturbance and changed little over time. Foraminifera showed insignificant mortality, and increased in abundance 10 days after the initial disturbance. Transport within the fluidized flocculent upper layer of sediment was probably the major dispersal mechanism in this community, and foraminiferans seem to be the least able to use this mechanism.

The meiobenthos of this habitat is described as a well-dispersed and dynamic community able to rapidly adjust to small-scale disturbances. However, the meiobenthos may not recover from all disturbances, because resilience was only determined for a limited physical disturbance.

## INTRODUCTION

Resilience of ecosystems, i.e., "the degree, manner and pace of restoration of initial structure and function in an ecosystem after disturbance" (Westman, 1978), has become a subject of growing importance in recent times. Ecologists have traditionally been interested in determining the role of disturbance in structuring communities (i.e., Dayton & Hessler, 1972; Cairns *et al.*, 1977; Connell & Slayter, 1977). The addition of the concept of resilience has produced new insights and interest in traditional approaches.

The rate of restoration of a community, "elasticity", sensu Westman (1978), is the most easily defined characteristic of resilience. Due to increasing technology and greater risk of catastrophic man-made disturbance, studies geared toward discovering the recovery rates of a variety of ecosystems are being actively explored (Cairns & Dickson, 1977, streams; Tompkins & Grant, 1977, grasslands; Christian *et al.*, 1978, microbial communities; Emerson & Zelder, 1978, and Murray & Littler 1978, marine algal communities).

The resilience of marine benthic systems has received a great deal of attention,

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but most of these studies have tried to assess the resilience of macrobenthic communities (e.g., Rosenberg, 1972; Kaplan *et al.*, 1975; Dauer & Simon, 1976; McCall, 1977; Simon & Dauer, 1977; Leppäkoski & Lindström, 1978; Rhoads *et al.*, 1978). Meiobenthic organisms, defined as those benthic micrometazoans and foraminiferans between 63 $\mu$m and 500 $\mu$m in size, have been studied much less intensively. Studies relating meiofauna and disturbances have primarily assessed the immediate effects of a disturbance, and not the resilience of the system (e.g., Bleakley & Boaden, 1971; Gray & Ventilla, 1971; Marcotte & Coull, 1974; Wormold, 1976; Cantelmo & Rao, 1978). The few meiobenthic studies to deal with resilience have provided conflicting results. Coull (1969) and Damodoran (1972) worked with natural disturbances and reported recovery times on the order of months. However, Pequegnat (1975) and Rogers (1976) investigated resilience of meiobenthos to dredging and reported incomplete recovery after 7 and 13 yr respectively.

The present study was conducted to experimentally test the response of meiobenthos to a disturbance. In our experimental design, disturbance consisted of turnover of sediment and production of anoxic conditions. The study addressed the following questions. (1) Does this proposed perturbation depopulate a meiobenthic community? (2) What are the rates of recolonization in the disturbed habitat, i.e., how long does it take for a disturbed meiobenthic assemblage to return to initial levels of density and diversity ("elasticity" of Westman, 1978)? (3) Are there taxa or species-specific responses to the disturbance? (4) Are there areal differences in the colonization process, i.e., does the center of the disturbed habitat get colonized more slowly than the edge? (5) Is colonization directional, i.e., does more colonization take place on incoming (ebbing) or outgoing (flooding) tides?

## Methods and Materials

A homogeneous, ovoid, intertidal mudbar (15 m × 6 m at the longest and widest portions respectively) in the upper regions of Crabhaul Creek, North Inlet, Georgetown, South Carolina, U.S.A. (32°20′N: 79°10′W) was selected as our study site. North Inlet is a well-mixed, high salinity estuary with numerous meandering tidal creeks bordered by *Spartina alterniflora* Loisel marsh with a mean tidal range of 1.3 m. The mud bar is covered by water approximately 7 h per 12-h tidal cycle, is 10 cm above MLW at its highest point and is covered by a flocculent detrital layer 0.5–1 cm thick. The upper 1.5–2 cm is oxidized.

Two 9-$m^2$ areas on the mudbar were marked with stakes (Fig. 1). One site served as a control area and one site, the disturbed area, was dug with shovels to a depth of 15 cm, turned over (to bring black anoxic mud to the surface), and raked smooth to approximate the microtopography of the control. Nine days prior to this sediment disturbance (referred to as Day −9 hereafter), three replicate 1.91-cm diameter cores were collected from the control and "disturbed" sites to establish pre-

disturbance densities and species composition, and insure that the sites were comparable.

Five stations (A–E) were established on the disturbed 9-$m^2$ area (Fig. 1). Station placement was designed to test for an edge effect in any possible colonization, i.e., would the center of this habitat (E) be colonized more slowly than the edge (A, B, C, D)? The 9-$m^2$ area was oriented parallel to the tidal current to test directionality of the colonization, i.e., would more colonization take place on ebbing or flooding tides?

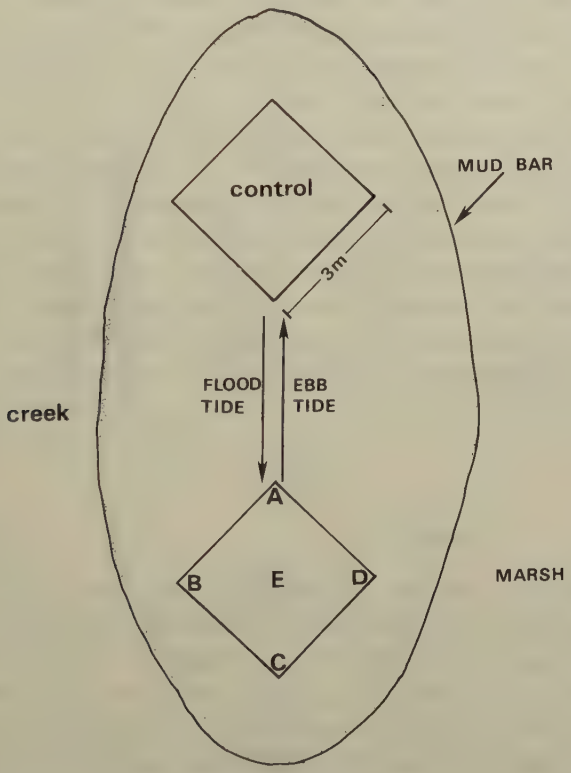


Fig. 1. Schematic diagram of the sampling site (mud bar) with disturbed and control plots.

Following disturbance, samples were collected at Days 0, ½, 1, 2, 3, 7, 10, 17, 24, 38, 52 and 80 (31 May–19 August 1978). Day 0 samples were taken 2 h after the sediment was overturned but before water covered the site, Day ½ samples were taken at the next low tide (midnight) and the remaining samples were taken at the daytime low tide. Three 1.91-cm internal diameter cores (4 cm deep) were collected from each of five disturbed sites and the control site (total of 18 cores) on each collection date. Samples from all the stations except E were obtained by leaning over the disturbed site from adjacent areas. Site E (center of disturbed site) was sampled

from a board placed between two supports on either side of the disturbed site. Salinity (measured by refractometer), air and water temperature (measured by hand-held thermometer) and diagrams of mammal and bird tracks and sightings of macrofauna on the sites were taken at each collection date. Samples for grain size and sediment organics were taken weekly and analyzed according to the methods outlined in Buchanan & Kain (1971).

In the laboratory animals were extracted from samples using sucrose flotation (Sikora, 1977) and passed through a 63-$\mu$m sieve. Material remaining on the 63-$\mu$m sieve was preserved in 10% buffered sea-water formalin, stained with Rose Bengal and sonicated (Thiel *et al.*, 1975). The samples from Day $-9$, 0, $\frac{1}{2}$, 2, 7 and 10 were sorted to major taxon. Nematodes and copepods were identified to species from samples for all days sorted except for Day 10.

Statistical analyses were performed using Sheffé's multiple comparisons method (Ott, 1977) using log ($\ln + 1$) transformation since variance was correlated with mean abundance. Comparisons were made on abundances of Nematoda, Copepoda, Foraminifera, other meiofauna taxa and total meiofauna at each site and at each time. This method, although producing somewhat conservative confidence intervals, has the advantage of allowing for the comparison of data in various combinations (i.e., control vs. A, B, C, E for the effect of disturbance, A, B, C vs. E for edge effect, etc.). Significance of all comparisons was determined at the $P \leqslant 0.05$ level.

## Results

### PILOT-TEST

To test the effect of a sediment disturbance by hand-turning, we initiated a pilot study in February, 1978. A 0.09-$m^2$ area was disturbed by digging 15 cm into the sediment and turning it over. Since only the uppermost 2–3 cm of these muds was oxidized, the fauna was placed 12–13 cm beneath anoxic mud. One hour later, in three cores taken 16 cm deep and sectioned at 2-cm intervals, no animals were alive, bacterial attack of all animals was obvious and there was no evidence of vertical movement of meiofauna from the displaced oxidized layer toward the surface. The preliminary data of the pilot study allowed us to hypothesize that the proposed major disturbance should cause almost total mortality of the meiofauna and that recolonization must occur horizontally, i.e., no colonization from vertically migrating meiofauna.

### TOTAL MEIOFAUNA

Fig. 2 illustrates the response of the total meiofauna assemblage, nematodes, copepods and foraminiferans to the major (May 1978) disturbance. Control and pre-disturbance sites were not significantly different in any category at Day $-9$.

At the time of the disturbance (Day 0) the fauna at the disturbed site declined precipitously (Fig. 2). Scheffé's multiple comparisons test found significant differences in control and combined disturbed sites for total meiofauna, nematodes, and cope-

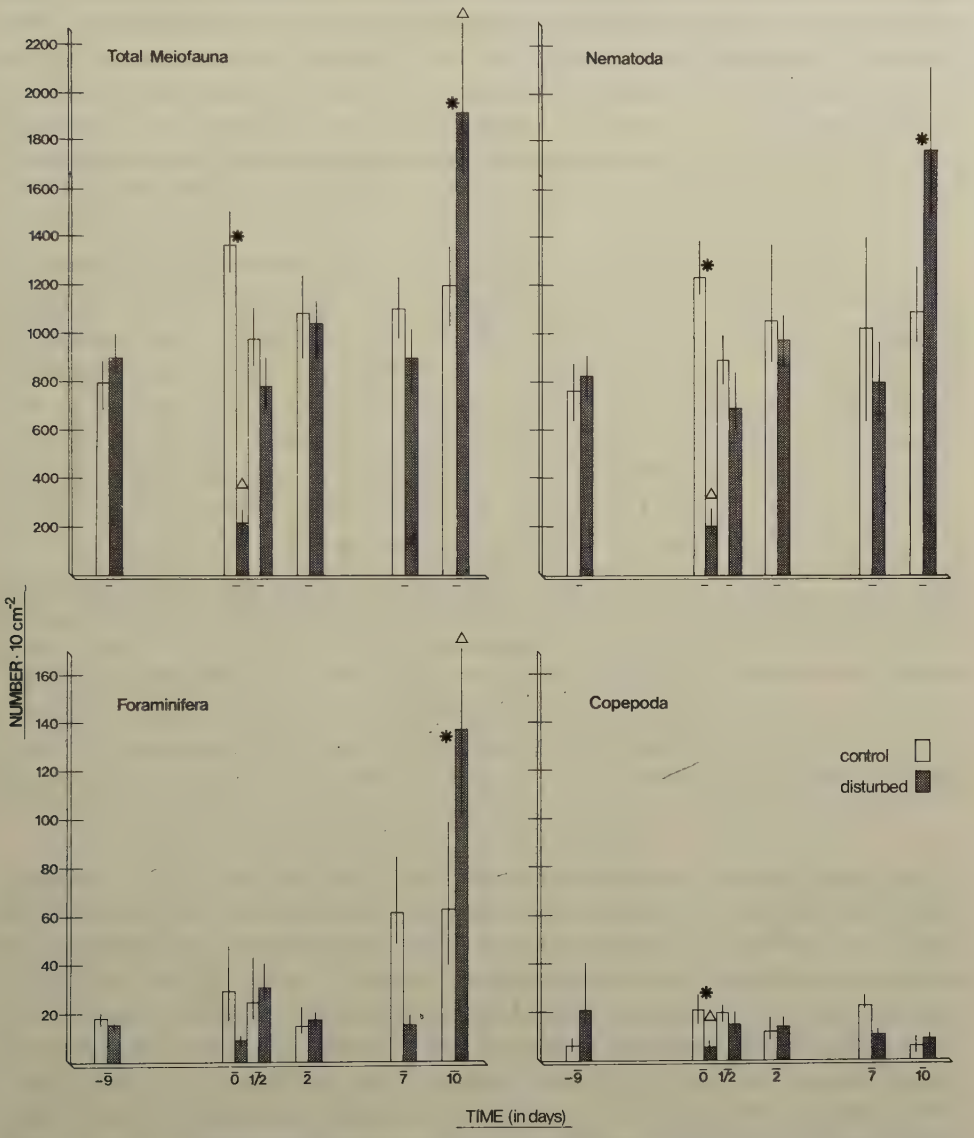


Fig. 2. Control and disturbed density values for total meiofauna, Nematoda, Copepoda and Foraminifera, prior to the disturbance (Day −9), immediately following the disturbance (Day 0), and after the disturbance (Days ½, 2, 7, 10): significant results were determined by comparing control and disturbed site values on the same sampling Day * and by comparing the disturbed site values at one time to the remaining sampling Days △ (control site showed no significant differences over time); SE bars (ln + 1 transformed) are plotted around the mean.

pods. Mortality was not absolute at this larger scale because the disturbance apparently did not displace all of the fauna into the redox layer, probably because sediments on the shovel edges were displaced only a few centimeters. Still, the entire surface of the disturbed site was black anoxic mud. Anoxia appeared to affect all taxa equally (Table I), although copepods experienced a slightly higher mortality (not statistically significant) than did nematodes and forams.

TABLE I

Mean densities (no. · 10 $cm^{-2}$) ± 1 SD of meiobenthic taxa on the site before (Day −9) and immediately after the disturbance (Day 0) on control and disturbed sites to illustrate the mortality caused by the disturbance.

| | Nematoda | Foraminifera | Copepoda |
|---|---|---|---|
| Day −9 | 835 ± 142 | 10.5 ± 2.8 | 25.6 ± 17.1 |
| Day 0 (control) | 1259 ± 134 | 28.4 ± 22 | 20.0 ± 9.2 |
| Day 0 (disturbed) | 250 ± 62 | 4.6 ± 2.0 | 2.9 ± 3.4 |
| Mortality (%) | | | |
| (a) Compared to Day −9 | 1 | 66 | 89 |
| (b) Compared to Day 0 (control) | 80 | 84 | 85.5 |

TABLE II

Mean densities (no. · 10 $cm^{-2}$) ± 1 SD of total meiofauna on the disturbed sites (A, B, C, E), Site D (sediment disruption without significant mortality) and the control site during the earliest stages of recolonization.

| | Sites A, B, C, E | Site D | Control |
|---|---|---|---|
| Day 0 | 224 ± 93.3 | 1112 ± 274.6 | 1360 ± 218.1 |
| Day ½ | 766 ± 173.9 | 885 ± 451.3 | 968 ± 175.4 |

Total meiofauna and nematode densities at Site D on Day 0 were equal to control levels, indicating the disturbance did not cause significant mortality at this site (Table II). Possibly the disturbance was ineffective here because the fluidity of the sediment prevented adequate displacement and establishment of anoxia; thus Site D cannot be considered a disturbed site. Scheffé's multiple comparison test found no significant differences in any taxa category (total meiofauna, nematodes, copepods, foraminiferans, and other meiofauna) between disturbed Sites A, B, C and E until Day 10 where Sites C and E were significantly higher in total meiofauna and nematodes than Sites A and B. From these results we were able to reject the hypotheses of an edge effect in colonization (E was never lower than ABC) and directionality (no differences A and C). Division of the disturbed habitat provided no additional information. To clarify Figs. 2 and 3 and for more accurate statistical comparisons, Sites A, B, C and E are collectively presented as the disturbed site.

The surprising resilience of the meiofauna in this experiment is well documented by the sharp rise in density of all taxa shortly following the disturbance (Fig. 2). Within one tidal cycle (Day $\frac{1}{2}$), density at the disturbed sites equalled predisturbance and corresponding control levels. This equality continued through the study until Day 10, when significant increases in total meiofauna, nematodes and forams occurred on the disturbed sites.

None of the observed changes can be related to changes in grain size or sediment organics. There were no significant differences in median grain size, sorting or sediment organics in any of the sediment samples processed.

### SPECIES PATTERNS

Although densities of meiofauna reached control and predisturbance levels rapidly (Fig. 2), species patterns provided additional insight into the colonization process. Fifty-two species of nematodes were collected during this study. *Ptycholaimellus pandispiculatus* Hopper was by far the most dominant ($\bar{X} = 163 \cdot 10$ cm $^{2}$); followed by *Tershellingia longicaudata* de Man ($\bar{X} = 70 \cdot 10^{-2}$); *Odontophora setosa* (Allgen) ($\bar{X} = 38 \cdot 10$ cm $^{-2}$); *Mesotheristus erectus* (Wieser & Hopper) ($\bar{X} = 33 \cdot 10$ cm $^{2}$); *Ptycholaimellus ponticus* Filipjev ($\bar{X} = 32 \cdot 10$ cm $^{2}$); *Metachromadora* (Metachromadoroides) *pulvinata* Wieser & Hopper ($\bar{X} = 24 \cdot 10$ cm $^{-2}$); *Theristus* sp. ($\bar{X} = 23 \cdot 10$ cm $^{2}$); *Adoncholaimus* sp. ($\bar{X} = 22 \cdot 10$ cm $^{2}$); and *Metachromadora* (M.) *remaneii* Gerlach ($\bar{X} = 21 \cdot 10$ cm $^{-2}$). These nine nematodes comprised 70% of all the nematode species collected. Fig. 3 illustrates these nine species patterns on the disturbed and control sites from Day 0 to Day 7. All species on the disturbed site have similar patterns, i.e., low values on Day 0, a subsequent increase and leveling off to control levels by Day 7. The patterns on the control site, however, were not the same for all species. Two species (*Ptycholaimellus pandispiculatus* and *P. ponticus*) had significantly lower control densities on Day $\frac{1}{2}$ (the only night sample), while all other species showed no significant differences in control densities over time.

Ten benthic copepods were identified during the study. *Paronychocamptus wilsoni* (Coull) was the dominant ($\bar{X} = 2.6 \cdot 10$ cm $^{2}$), followed by *Nannopus palustris* Brady ($\bar{X} = 1.6 \cdot 10$ cm $^{2}$); *Stenhelia* (D.) *bifidia* Coull ($\bar{X} = 1.5 \cdot 10$ cm $^{2}$); *Enhydrosoma propinquum* Brady ($\bar{X} = 1.2 \cdot 10$ cm $^{2}$) and *Cyclopoid* sp. ($\bar{X} = 0.6 \cdot 10$ cm $^{2}$). These five species comprised 84% of all the copepods. Since the site was so overwhelmingly dominated by nematodes and individual copepod densities so low, the only significant ($P \geqslant 0.05$) pattern was a decrease in the five species at Day 0 on the disturbed site. After Day 0 there were no significant differences in any species over time or between the disturbed and control areas.

## DISCUSSION

After significantly reducing meiofaunal density by sediment disturbance (Fig. 2), we have demonstrated that meiofauna are capable of recolonizing the disturbed

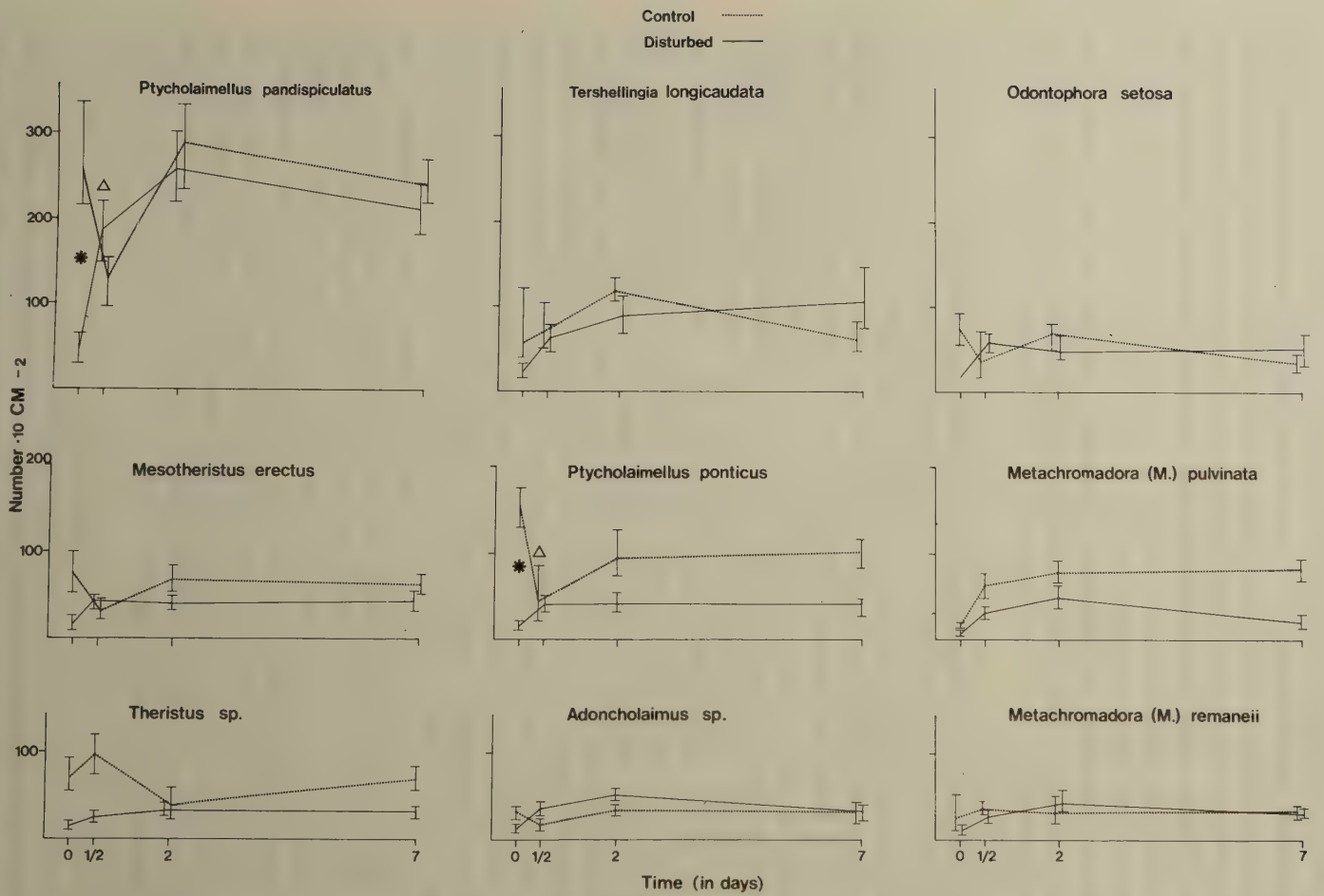


Fig. 3. Densities of the nine dominant nematode species on the control and disturbed sites on Days 0, $\frac{1}{2}$, 2 and 7: symbols as in Fig. 2; SE bars (ln + 1 transformed) are plotted around the mean.

area (9 $m^2$) within one tidal cycle. At the major taxon level all taxa increased to control and/or predisturbance densities within 12 h of the disturbance. There was no evidence that recolonization was directional or related to stage of the tide.

The recolonization rates presented here are the fastest known for meiofauna. Coull (1969) and Damadoran (1972) reported that it took months to recolonize defaunated areas, while Pequegnat (1975) and Rogers (1976) report times in years. The last two studies may not be comparable to ours because there was repeated disturbance (maintenance dredging) in the first study, and no assurance that the control and disturbed sites were originally similar in the second. Experimental studies on colonization of meiofauna (Boaden, 1969; Nair & Govindankutty, 1972; Scheibel, 1974; Conrad, 1976) are not comparable to our study. These researchers did not attempt to quantify rates of colonization; rather, they studied whether colonization took place at all, and/or what effect sediment size or angularity had on the composition of the colonizing fauna. In all these experimental studies, however, colonization had started by the time of the first "after" sample. As examples, Boaden (1969) took his first samples 20 days after placing his graded sands in the habitat and found that meiofauna were present (different densities and species dependent on type of sand colonized). Scheibel (1974) found meiofauna in his azoic sediment boxes 3 days after establishment (his first sample). Our data cause us to wonder what would have happened if Boaden or Scheibel had sampled $\frac{1}{2}$ day after their azoic sediments were placed in the environment – would they have been colonized? Recolonization of the order of hours, as we have demonstrated, suggests that the meiofauna may be much more mobile than previously thought.

The Foraminifera pattern, i.e., no significant density differences at Day 0 between control and disturbed sites, and subsequent increase in foram abundance on Day 10, is anomalous when compared to the other meiofauna taxa patterns. We suspect that the absence of a drop in abundance on Day 0 may be an artifact since non-living foram tests covered with bacteria stained with Rose Bengal and were indistinguishable from living forams. Therefore, living specimens were probably overestimated in Day 0 samples. The dramatic increase in Foraminifera abundance on Day 10 (Fig. 2) on the disturbed site may be linked to their poor dispersal powers, thus the relatively late establishment of a foram assemblage. Forams, with their heavy calcium carbonate tests, are probably less easily suspended in the water column and active migration would be slower than for other taxa. Wefer & Richter (1977), working with azoic sediment colonization by forams in Kiel Bay, found colonization started 3–5 months after the start of the experiment. Compared to Scheibel's (1974) azoic sediment experiment, forams seem to be the least mobile members of the meiofauna.

The significantly lower control densities of the nematodes *Ptycholaimellus pandispiculatus* and *P. ponticus* (Fig. 3) on Day $\frac{1}{2}$ are enigmas. Since Day $\frac{1}{2}$ was the only night sample set, perhaps there was movement (passive or active?) of these two species at night. Of course, it is impossible to base too much conviction on but one night sample period even if meiofauna are known to undergo diurnal vertical migra-

tion (Boaden & Platt, 1971; Dye, 1978). At our sites, vertical migration seems unlikely since only the upper 1–3 cm of sediment was oxidized and obviously a repetitive day–night sampling scheme is needed to test for any light controlled movement.

The mode of the rapid (one tidal cycle) recolonization is impossible to delineate and two alternatives exist: 1) active migration by crawling, or 2) passive transport with the tide. We hypothesize that most of the recolonization was horizontal via passive transport with the tide because it is unlikely that such small animals could crawl such large distances within one tidal cycle. Current velocities at the mouth of the experimental creek are available for four sampling periods spanning several seasons. The mean current velocity at the mouth of the creek, including periods when the creek was completely drained, was 15.5 $cm \cdot s^{-1}$ (range 0–60; $N = 77$) (L. R. Gardner, pers. comm.). Using the mean current velocity, the boundary shear stress at the sediment–water interface was calculated following Sternberg (1972) by $\tau_0 = 3 \times 10^{-3} p\bar{U}_{100}^2$; where $\bar{U}_{100}$ = current velocity at 1 m above the surface and $p$ = water density constant, 1.03 $g \cdot cm^{-3}$. Even the mean shear stress of 0.735 $g \cdot cm^{-3}$ is capable of moving quartz sediment particles (density of 2.65 $g \cdot cm^{-3}$) and, thus capable of moving 0.035- to 0.080-mm diameter nematodes (Levy & Coull, 1977), with a density of 1.13 $g \cdot cm^{-3}$ (Weiser, 1960). Certainly once velocities and shear stress exceed the mean, nematodes could be moved even if they are not truly representative of a smooth sphere (the assumption on which the calculations are made).

Calcareous Foraminifera have a density of 1.7 $g \cdot cm^{-3}$ (density of calcite, assuming protoplasmic density of 1) and Kontrovitz *et al.* (1978) report that current velocities of 5.1–18.7 $cm \cdot s^{-1}$ are necessary to move an empty foram test, depending on weight and sphericity. Since the forams we encountered were large, alive, and could adhere to sediment particles with the pseudopodia, one might expect much higher velocities would be necessary to move them, and, thus passive movement to be very slow to non-existent. We suspect that this is the reason foram recolonization did not occur as quickly as with the other taxa. Since all the meiofauna at and near the disturbed site reside in the upper 1–2 cm of surface (most of which is detrital floc and constantly in motion; personal observation), the non-foram meiofauna could easily be transported within this flocculent boundary layer. Furthermore, preliminary experiments of freezing cores and the overlying water in situ at the same site (Bell & Sherman, unpubl. data), indicate that large numbers of meiofauna occur in the overlying water. Thus, we feel that recolonization most likely took place by horizontal transport by the overlying water.

At least two alternatives exist as to how the fauna occupied the disturbed area so quickly and reached and maintained densities at the control levels: (1) the disturbed sites represented uninhabited space, i.e., an open resource, or (2) we changed the sediment characteristics (e.g., bacteria, diatoms), thus making the disturbed site more "attractive" to the meiofauna. We suspect that the hypothesis of open resource (no. 1) is the more reasonable assumption of the two and our arguments are based primarily on the disturbed sub-site that did not show significant mortality.

We mentioned earlier (see p. 64) that Site "D" showed no significant mortality after the sediment disturbance, and, thus, we excluded it from our results. However, Site D on the disturbed 9 $m^2$ can be considered a separate treatment, i.e., sediment disturbance without meiofauna mortality. We, therefore, had three experimental treatments: (1) no disturbance (control); (2) disturbance and mortality (Sub-sites A, B, C, E on the disturbed site) and (3) disturbance and no mortality (Sub-site D). Had sediment disturbance been the primary factor encouraging colonization, we would have expected Site D to experience an increase in density at Day $\frac{1}{2}$ (an additive effect) beyond the control and/or disturbed site levels. Since sub-site density values at Day $\frac{1}{2}$ were not significantly diferent than Sub-sites A, B, C and E or the controls (Table II), it appears that the sediment disturbance itself did not make the disturbed sites more attractive. Furthermore, because there were no significant differences in any sites after Day 0, and density values levelled off very quickly, recolonization was most likely the result of occupying uninhabited space. Once densities reached "saturation" levels (a density effect), colonization was balanced with emigration and density was maintained. It appears, then, that there was a maximum density the meiofauna could obtain – they did so very quickly (one tidal cycle) and then maintained this homeostatic density level throughout our experiment. The disturbed site, however, shows that disturbances may alter this pattern (Fig. 2, Day 10). It is not known if this density increase on the disturbed site is a temporary fluctuation between control and disturbed sites or if it persists over the later sampling periods.

We have proposed a conceptual framework for the meiofauna of an intertidal mud region as a dynamic, highly dispersive assemblage. The animals of this region are intimately associated with the overlying water column, and may use it, along with active migration and selection of habitats to be dispersed at least in areas with substantial near-bottom water movement. Dispersal may be an important, often unrecognized, structuring influence in this community, since meiofauna can rapidly respond to small-scale disturbances and return them to an undisturbed state quickly. Other, more natural disturbances, such as those caused by bottom feeding of fish, sediment disruption by rays or skates and footprints of researchers may provide unoccupied habitats for exploitation by meiofauna. Our work reemphasizes this adaptation of meiofauna to such biological and physical disturbance.

We have demonstrated rapid recolonization rates of meiofauna for a limited, small-scale disturbance. It would be unwise to generalize that meiofauna recover quickly from any or all disruptions of their environment. Larger, more catastrophic or repeated disturbances may result in much slower recolonization rates. The resilience of an ecosystem is dependent on the type of disturbance and certainly these data on meiofauna recolonization rates indicate that these omnipresent organisms should become the subject of more studies designed to test resilience in benthic ecosystems.

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# A LABORATORY INVESTIGATION OF THE EFFECT OF A TEREBELLID POLYCHAETE ON THE SURVIVORSHIP OF NEREID POLYCHAETE LARVAE

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**Abstract:** A controlled laboratory experiment showed that the survivorship of the larvae of *Nereis vexillosa* Grube is significantly lowered by the feeding activities of *Eupolymnia heterobranchia* (Johnson). Ingestion of larvae by *Eupolymnia* is suggested as the mechanism of this interaction. *E. heterobranchia* eats small metazoans in the laboratory and also has microcrustacean and polychaete hard parts in its feces. These findings support the hypothesis that adult–larval interactions are important in structuring dense marine infaunal assemblages. It is argued that such interactions are important in other types of communities, e.g., terrestrial forest and marine fouling communities, when they are at or near their resource limitation.

Biological activities may be largely responsible for the maintenance and persistence of dense assemblages of infaunal organisms. Such biological activities which are regarded as important are the occupation of space to the exclusion of other species or functional groups (Mills, 1967; Daro & Polk, 1973; Woodin, 1974; Ronan, 1975; Peterson, 1977; Brenchley, 1978), the provision of refugia in the form of biologically created structures (Orth, 1977; Reise, 1978; Woodin, 1978), and the biologically mediated death of settling larvae either through direct consumption or indirect sediment effects (Rhoads & Young, 1970, 1971; Woodin, 1976; Myers, 1977; Brenchley, 1978). In this paper, I examine the importance of the last category of interactions in a controlled laboratory experiment.

Woodin (1976) presented theoretical arguments and inferential data to show that interactions of established adults with settling larvae are important determinants of community structure in dense infaunal assemblages. However, no direct tests of the predictions of the hypothesis have been published. Field experiments designed to document this phenomenon are fraught with difficulties because factors, such as (1) immigration of adults and settled juveniles through the sediment, (2) strong habitat selection of settling larvae (Scheltema, 1974), and (3) temporal and spatial variation in larval abundance, may obscure the predicted reduction of recruitment found in the presence of high adult densities. I resorted, therefore, to a laboratory experiment where the effects of the feeding activities of the terebellid polychaete, *Eupolymnia heterobranchia* (Johnson), on the survivorship of larvae of the nereid polychaete, *Nereis vexillosa* Grube, were tested.

## Study Organisms

*Eupolymnia heterobranchia* is perhaps the most common terebellid in soft-sediment systems in the San Juan Archipelago, Puget Sound, U.S.A. Attaining lengths of 12 cm, it lives in a mucus-lined U-shaped tube made primarily of large sediment particles and shell fragments. Each end of the tube has a chimney which projects about 1 cm above the sediment surface. Small *E. heterobranchia* may build a tube on pieces of the algae, *Ulva* and *Monostroma*, although the largest individuals are infaunal. *Eupolymnia heterobranchia* feeds in the field as a typical terebellid (Fauchald & Jumars, 1979) by spreading its tentacles over the surface of the sediment. When its tube is covered by $<15$ cm of water, I have observed this species in the field to spread the distal portions of its tentacles at the air–water interface which permits the proximal portions of the tentacles to gather suspended matter from the water column. The species will also feed in the water column by resting the proximal portions of its tentacles on the tube chimney.

*Nereis vexillosa*, a herbivore (Woodin, 1977), occurs in the same habitat as *Eupolymnia heterobranchia*. *Nereis vexillosa* is unusual among nereid polychaetes in that females lay egg masses which are often 8 cm across and contain hundreds of eggs (Johnson, 1943). These egg masses are not attached to the substratum but are buffeted and transported by tidal currents. The larvae normally hatch as 3-setiger larvae and remain in the plankton until the 5- or 6-setiger stage is attained. The larvae feed on endogenous yolk globules and later on phytoplankton (Johnson, 1943). The egg masses can be used to obtain large numbers of similarly-sized larvae for experiments such as these reported here.

## Materials and Methods

To test the effect of the biological activities of *Eupolymnia heterobranchia* on the settlement and survivorship of *Nereis vexillosa* larvae, experimental chambers were constructed from plastic trays ($23 \times 15 \times 5$ cm deep). One or more pairs of holes were burned in the bottom of each tray. The ends of 15-cm pieces of Tygon tubing (either 11 or $12\frac{1}{2}$ mm in diameter) were wedged up through the holes in the bottom of the trays to simulate natural *Eupolymnia heterobranchia* tubes. Each end of the tubing projected about 1 cm above the bottom of the tray. Sediment from the *E. heterobranchia* collection site was placed in fresh water for 9 days to kill all the marine organisms and subsequently inoculated for 1 day with filtered sea water. Fifteen $cm^3$ of this sediment was placed into each experimental tray along with 500 ml of filtered sea water. The trays were floated in a sea-water table at the Friday Harbor Laboratories, Washington, U.S.A.

*E. heterobranchia* and *Nereis vexillosa* egg masses were collected from Mitchell Bay on San Juan Island, Washington (see Woodin, 1974 for map of the area).

Worms were introduced into the Tygon tubes according to the experimental treatment assigned to each tray. Most *Eupolymnia heterobranchia* readily adopted the Tygon tubes, although during the course of the experiment some vacated the Tygon tubes and built their own sediment tubes along the bottom edge of the tray. Worms which vacated their Tygon tubes showed no difference in feeding activity from those which adopted the artificial tubes. Worms in the artificial tubes coated the inner portion with mucus and sediment, often constructing a sediment chimney. *E. heterobranchia* easily turns around in its tube and thus can feed from either opening. After the *E. heterobranchia* had been introduced and established themselves, 180 *Nereis vexillosa* larvae were introduced into each experimental tray. The larvae were mostly 3-setiger stages which had been isolated 1 or 2 days prior to the beginning of the experiment. Each tray received approximately equal aliquots of larvae which had been isolated for 1 and 2 days.

There were six treatments in the experiment, each replicated three times. Three treatments, designated 1X, 2X, and 3X, received one, two, and three *E. heterobranchia*, respectively, corresponding to densities of 29, 58, and 87 worms/$m^2$. A Tygon tube control (TC) tested survivorship of larvae in the presence of Tygon tube material without any terebellids. Another control (C) tested the survivorship of larvae in the absence of both terebellids and Tygon tubes. Finally, a sediment control (SC) was utilized in which two *E. heterobranchia* were introduced and allowed to feed for 4 h, thus restructuring the sediment. This control was designed to distinguish between (1) no effect by *E. heterobranchia* on settlement of *Nereis vexillosa* larvae, and (2) induction of settlement resulting from sediment restructing by the terebellids and subsequent ingestion of the *N. vexillosa* larvae. Both of these possibilities could produce the same pattern.

The experiment was terminated after 3 days. The water in each tray was decanted off through a 64-$\mu$m sieve and material retained was searched for "planktonic" larvae under a dissecting microscope (25 ×). The sediment was placed in 10% seawater formalin with Rose Bengal. The total volume of the *Eupolymnia heterobranchia* in the 1X, 2X, and 3X treatments was determined by displacement in a 50-ml graduated cylinder. Sorting of the sediment for larvae was done under a dissecting microscope (12–25 ×). Differences in larval survivorship in the various treatments were tested for significance by a one-way analysis of variance after applying a log ($x$) transformation to the data to correct for heterogeneity of variance. A one-way analysis of covariance was performed on the survivorship data for the 1X, 2X, and 3X treatments to test for size-dependent effects. These data were also log ($x$) transformed.

To attempt to see actual ingestion of larvae by *E. heterobranchia*, some *E. heterobranchia* were established in sediment-filled beakers in Tygon tubes. A beaker was placed in a plastic aquarium through which water from the sea-water system was allowed to flow. A dissecting microscope mounted on a swing arm stand permitted observation of feeding from above. Light was provided by a fiber optic system.

*Nereis vexillosa* nectochaetae were introduced on the sediment or tube chimney and interactions observed.

Fecal material of *Eupolymnia heterobranchia* was examined from specimens collected in Mitchell Bay which were promptly returned to the laboratory and isolated in plastic Petri dishes. The fecal material was collected and preserved in 10% seawater formalin. The feces were later examined under a compound microscope.

## Results

Fig. 1 presents data from the *Eupolymnia–Nereis* larvae experiment. The ANOVA indicates that there are significant differences among treatments ($F_{5,12} = 17.23$, $P < 0.001$). A posteriori Student–Newman–Keuls test (Sokal & Rohlf, 1969) (Table I) indicates the differences in survivorship of *N. vexillosa* larvae as follows: $3X \ll 2X < 1X = SC = TC = C$, where $\ll$ indicates $P < 0.01$, $<$ denotes $P < 0.05$, and $=$ indicates no statistical difference. From Table I, the only exception to the

Fig. 1. Number of larvae surviving to end of experiment: initially 180 larvae were introduced into each container; mean and standard deviations are plotted; each treatment is replicated three times; C, container control; TC, Tygon tube control; SC, sediment modification control; 1X, 2X, 3X, treatments with one, two, and three *Eupolymnia heterobranchia* per tray.

Table I

Results of Student–Newman–Keuls tests for significant differences between treatments: means which are not significantly different ($P > 0.05$) are underlined.

| Treatment | 3X | 2X | SC | 1X | TC | C |
|---|---|---|---|---|---|---|
| Mean | 16.7 | 40.3 | 61.3 | 76.7 | 66.7 | 66.7 |

hierarchy given is the 2X and SC treatments which are not significantly different. This equivalence is probably an artifact of the small sample size and associated high variance (Fig. 1) but may indicate a secondary sediment effect. When the *Eupolymnia heterobranchia* volumes are added to the analysis as a covariate, the ANOCOVA indicates the covariate is not significant ($F_{2,6} = 3.0$, $P > 0.05$). Thus there is a density-dependent but no size-dependent effect of *E. heterobranchia* on *Nereis vexillosa* larval survivorship. The highest density of *Eupolymnia heterobranchia* used in this experiment ($87/m^2$) is less than the average density of $180/m^2$ given by Woodin (1974) for Mitchell Bay. One therefore predicts even stronger effects in the field than those seen in the laboratory.

The experiment established that *E. heterobranchia* activities represent a significant mortality source for *Nereis vexillosa* nectochaetae in the laboratory. To demonstrate that actual consumption of larvae is the mechanism of this interaction, I attempted to observe ingestion of larvae in the laboratory. The small size of the larvae necessitated observation at 25–50 × affording a very small field of view. As a consequence, I was not able to directly observe the capture and ingestion of larvae although I did observe the capture of *Corophium* sp. by a coordinated effort of many tentacles and subsequent ingestion of the amphipod.

Additional supporting data were gathered by examining fecal material from three *Eupolymnia heterobranchia*. Sediment grains and diatom frustules were the major fecal constituents. However, the feces also contained two setose appendages of a small crustacean, a copepod nauplius, the remains of an amphipod, an unidentifiable crustacean exoskeleton, and the following polychaete setae: four smooth, capillary setae, one simple, spinous seta, and one falcigerous compound seta. It is not possible to determine if these prey items were captured or merely ingested as dead or moribund individuals.

## Discussion

Many infaunal assemblages exhibit patchiness of species and/or functional groups in the absence of any physical gradients (e.g., Woodin, 1976; Brenchley, 1978). Because these patchy assemblages are persistent, it is clear that recruitment into existing patches is rather low. Three classes of phenomena can produce this pattern: (1) habitat selection or gregarious settlement by competent larvae (see Scheltema, 1974), (2) sediment-mediated death of settling larvae or juveniles, and (3) mortality of larvae through ingestion by established adults. The first processes are species-specific ones; that is some species have highly selective larvae and others discriminate settlement sites little if at all. The second process is dependent on the functional groups involved. Brenchley (1978) showed that juveniles of sediment stabilizers were far more sensitive to suffocation by reworked sediments than the juveniles of sediment destabilizers (corresponding to the deposit-feeders of Rhoads & Young, 1970).

The third process, the concern of the present contribution, is of the most general applicability. Larvae should suffer mortality irrespective of species or functional group. All three of these phenomena should occur in dense infaunal assemblages with the first processes acting as a primary filter and the latter two classes of interactions acting concurrently as a second filter. All three of these phenomena are density dependent. Larvae which preferentially settle near conspecifics should have higher settlement in the presence of large numbers of adults (but see Connell, 1961, for inhibition of settlement above a critical density). Conversely, little settlement should occur of larvae in areas where there are few or no established conspecifics. In the second case, high densities of one functional group, e.g., sediment stabilizers, tend to have a greater negative effect on other functional groups than on their own functional group (Woodin, 1976; Brenchley, 1978; Woodin & Jackson, 1979). The last case is density dependent simply because the more established animals there are, the greater the total ingestion of larvae. The effect will be greater where organisms do not maintain strict feeding territories. Two species of *Eupolymnia* illustrate this point. Individuals of *E. crescentis* maintain circles of influence of $\approx 12$ cm in diameter. When tentacles of individuals touch, both tentacles mutually withdraw (Ronan, 1977). Individual *E. heterobranchia*, however, do not withdraw their tentacles when feeding areas overlap (pers. obs.). One therefore predicts greater consumption of larvae by *E. heterobranchia* than its congener, *E. crescentis*. One should see a pattern of increasing larval survivorship as distance from the adult tube increases. With *E. crescentis*, larval survivorship should vary as the square of the distance since the area of the feeding circle $\alpha$ distance$^2$. The pattern with *E. heterobranchia* should be more obscure since areas of influence overlap. However, migration of settled individuals through the sediment, local heterogeneity, and stochastic events will tend to blur the predicted patterns in the field.

Ronan (1977) showed that *E. crescentis* ingested small metazoans, some of which passed through the gut and were still motile when released from the fecal pellets. It is not clear if these organisms could escape from the pellets before suffocating. Miliekovsky (1974) showed that pelagic larvae which passed alive through benthic invertebrates generally died soon after. Ronan (1977) suggested that 30% of such larvae and small metazoans are killed and partly digested. Deposit-feeders then apparently ingest more than sediment and detritus. Although the contribution of metazoans to deposit-feeders' diets is small, such consumption can have a considerable impact on community composition. The results of the laboratory experiment (Fig. 1) attest to the importance of such ingestion of larvae, particularly since the highest experimental density was lower than natural densities. Whether one considers ingestion of larvae predation (some at least are digested) or a competitive interaction, the important point is that established adults reduce larval survivorship.

The experiment herein described clearly demonstrates that the presence of established adults in soft-sediment systems can affect the survivorship of larvae attempting to settle. This class of interactions must be important in other com-

munities as well. Standing (1976) has shown the hydroid, *Obelia dichotoma*, inhibits the settlement of barnacle cyprids. Harper (1977) cites several examples of even-aged stands of trees causing the mortality of newly germinated seeds or seedlings by shading or nutrient depletion. Interactions of established adults with propagules attempting to enter a system are probably an important community structuring process when resource limitation occurs.

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# THE RELATIONSHIP OF SAND MOISTURE TO BURROWING DEPTH OF THE SAND-BEACH ISOPOD *TYLOS PUNCTATUS* Holmes and Gay

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**Abstract:** The daytime distribution of *Tylos punctatus* Holmes and Gay was examined by coring on a sand beach in the northern Gulf of California. Moisture and temperature measurements in the cores indicated that the isopods burrow downward to reach sand with at least 1% moisture. Animals experimentally confined to sand with 0.1% moisture showed high mortality rates. We suggest burrowing depth is primarily controlled by a requirement for saturation humidity in the surrounding sand.

## INTRODUCTION

Adaptations for moisture conservation are a major factor in the evolutionary transition from marine to terrestrial habitats by crustaceans (Brusca, 1966; Edney, 1968). Terrestrial isopods (Oniscoidea) show varying degrees of morphological and physiological adaptations for reducing moisture loss through the cuticle, but all species that have been studied are critically dependent on behavioral mechanisms to locate humid microhabitats for at least part of every day (Gunn, 1937; Waloff, 1941; Cloudsley-Thompson, 1956; Warburg, 1964; Edney, 1968).

*Tylos punctatus*, an oniscoid isopod, inhabits the upper intertidal zone of sand beaches on the Pacific coast of North America from Santa Barbara, California south to Punta Baja, Baja California Norte (Hamner *et al.*, 1969) and in the northern Gulf of California south to Guaymas, Sonora (Van Name, 1940 in Schultz, 1970). It has been suggested that the Pacific coast animals are taxonomically distinct from those in the Gulf of California (Hamner *et al.*, 1969; Hayes, 1977), but formal descriptions to establish this have never been published. Both Schultz (1970) and R. Brusca (Allan Hancock Foundation, University of Southern California, Los Angeles, California), who has compared series of specimens from both regions (pers. comm.), do not recognize such a difference. In addition, Schultz (1970) refers to *T. punctatus* in all of Mulaik's (1960) references to *T. latreillei* (a Mediterranean–Atlantic–Caribbean species) from the western coasts of North America.

The ecology and behavior of *T. punctatus* have been studied along the Pacific

coast (Hamner *et al.*, 1968, 1969; Hayes, 1977), and the results suggest that several aspects of their behavior are related to moisture conservation. *T. punctatus* occur in patches above the high tide line on sand beaches and in burrows as deep as 60 cm during the day; and they emerge nightly to forage on beach wrack (Hamner *et al.*, 1969). Before re-burrowing each morning, the animals locate burrowing sites immediately above the previous high tide line by moving upslope on wet substrata and downslope on dry ones (Hamner *et al.*, 1968). This behavior may prevent the animals from being washed away during the next high tide and also may keep them within range of their food and moist sand (Kensley, 1972).

Kensley (1974) suggested that *T. grannulatus* in South Africa (ecologically similar to *T. punctatus*) burrows downward far enough to remain in moist sand while the surface layer dries out during the day. Hayes (1977) examined the relationship of abundance of *T. punctatus* to sand moisture content in cores from two beaches and found conspicuous differences between the two localities; he suggested that the isopods may have specific moisture preferences, but these preferences might be different on different beaches. He apparently measured the moisture content of the complete 20 cm deep cores used without differentiating between levels within the cores. We feel that moisture gradients with depth may have produced the apparent differences in selectivity reported by Hayes.

In this study we made a preliminary comparison of the depth distribution of *T. punctatus* Holmes and Gay to moisture levels over the relatively small scale of 5-cm depth intervals in order to determine the possible relationship of burrowing to moisture. Our study site was in the northern Gulf of California where moisture gradients may be of particular importance because of the dry desert climate with exposure to searing solar heat on the beach surface during summer days.

## Methods and Materials

Our studies were conducted at "Station Beach", about 3 km southeast of the town of Puerto Peñasco, Sonora, Mexico (near the University of Sonora/University of Arizona Marine Station).

The daytime depth distribution of buried *T. punctatus* was determined by coring along transects laid out perpendicular to the waterline, following the methods of Hamner *et al.* (1969). Using a 12.7-cm diameter pipe with a serrated lower end, cores were taken at 1-m intervals along the transects beginning just seaward of the line wetted by the most recent high tide and continuing landward. At each sample site, cores 2 cm and 5 cm deep (designated as 0–2 and 0–5 cm, respectively) were taken side by side; then additional 5-cm deep cores were taken at the site of the 0–5 cm core. This process continued downward until results showed that the zone of buried animals had been passed.

The animals were extracted from the sand by dumping the cores into a bucket of

sea water, agitating it vigorously, and pouring the supernatant liquid with included buoyant animals through a 2-mm mesh screen.

As each core was taken from the beach, sand temperatures were measured by implanting a laboratory thermometer 2 cm into the cavity wall (near the bottom) while keeping it shaded from direct sun. About 100 g of sand was collected from the side of the hole at the level of each 5-cm core; the samples were sealed in plastic bags and carried to Tucson. Then sand samples were weighed to the nearest mg, oven-dried for at least 6 h at 90–100 °C, and then reweighed for determination of moisture content (weight-loss divided by wet weight, expressed as percent).

Two transects were sampled for this study, one on 16 October, 1977 and the other on 29 October, 1977.

## Results

The horizontal distribution of *T. punctatus* on the two sample days was comparable to that reported by Hamner *et al.* (1969) on the Pacific coast. The highest concentration of animals appeared in cores 1–8 m upslope from recent high water marks on the beach (Figs. 1, 2). Both transects were sampled during times of diminishing spring tides (16 October, 4 days after a new moon, and 29 October, 3 days after a full moon). High winds during the penultimate extreme high tide on 29 October had carried waves far up the beach and resulted in a second, upper band of animals concentrated about 7 m higher on the beach than the primary band related to the most recent high tide.

Temperature measurements from the cores showed that on 16 October a steep temperature gradient (3–11 °C change) extended from the surface down to the 10 cm level (Fig. 1). The 0–2 cm samples were analyzed to obtain data on surface conditions which are not reflected in the larger 0–5 cm cores and indicated that the highest temperatures occurred only within the upper few cm. This transect was sampled during hot, calm weather, which accounted for the high surface temperatures.

The 29 October transect showed quite different temperature conditions: there was only a 1–2 °C change between the surface and the 25-cm level (Fig. 2). We attributed the low temperatures and lack of change with depth to overcast and windy weather occurring on this date.

The sand moisture analysis showed that at some sites in both transects the sand was relatively dry (less than 0.5% moisture) only near the surface, as seen in the 0–2 cm cores at meters 16–20 on 16 October (Fig. 1) and meters 17–21 on 29 October (Fig. 2). At other sites, dry sand extended as deep as the 0–5 cm cores and at one site (meter 15, Fig. 2) to the 5–10 cm core. In general, sand moisture varied in a highly irregular way between 1.1 and 4.0% at depths $>5$ cm (Figs. 1, 2). Despite the clear difference in temperature profiles for the two transects, no difference in moisture patterns was detected that could be attributed to the temperature differences between the two transects.

Animals were rarely found near the surface (0–2 cm cores), but were usually first encountered in the 0–5 and 5–10 cm cores, corresponding to the first occurrence of moisture levels greater than ≈1% (Figs. 1, 2). In both transects most of the animals were found between depths of 5 and 20 cm, inhabiting less range in depth than reported previously by Hamner *et al.* (1969). Comparing the occurrences of *T. punctatus* in the two transects to depth and moisture, we observed no general differences between the two sampling dates.

When animal abundance is plotted against sand moisture for the two sampling

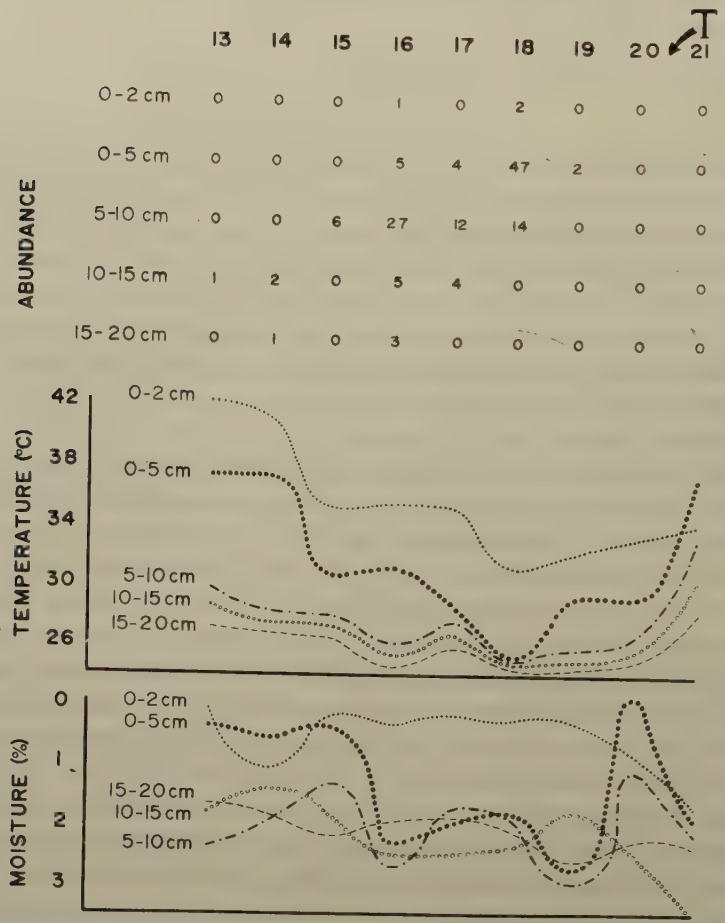


Fig. 1. The horizontal and vertical distributions of *Tylos punctatus* in the 16 October transect (number per core) are shown in the rows and columns above: depths of cores are designated by their range from top to bottom on the left side; the row of large numbers at the top indicates the distance in meters from the reference stake at the upper end of the transect; T, the highest level reached by the previous night's high tide; below, temperature and moisture distributions within the cores are plotted on the same horizontal axis as animal abundance.

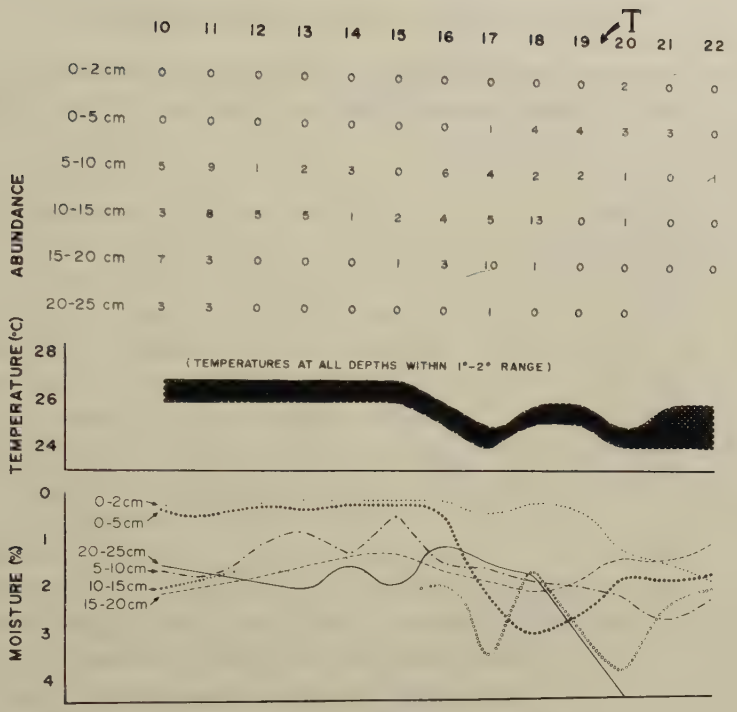


Fig. 2. The distributions of animals, temperatures, and moisture on 29 October, plotted similarly to Fig. 1.

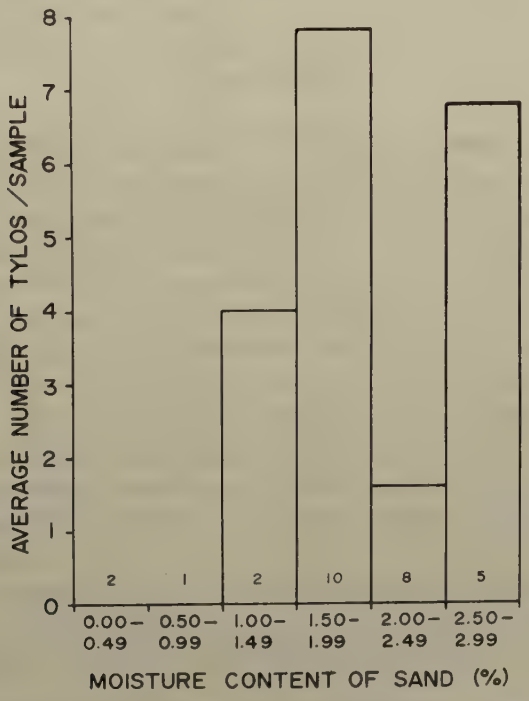


Fig. 3. The average number of *Tylos punctatus* per core within moisture intervals for the 16 October transect: the number of cores found within each moisture interval is shown within the columns; data from the 0–2 cm cores were not used because of their smaller volumes.

days (Figs. 3, 4), it is seen that most were in the intermediate range of 1.0–2.5%, with peaks at 1.5–2.0% for each transect. Sand with moisture >3.0% was rarely encountered within the area inhabited by the isopods.

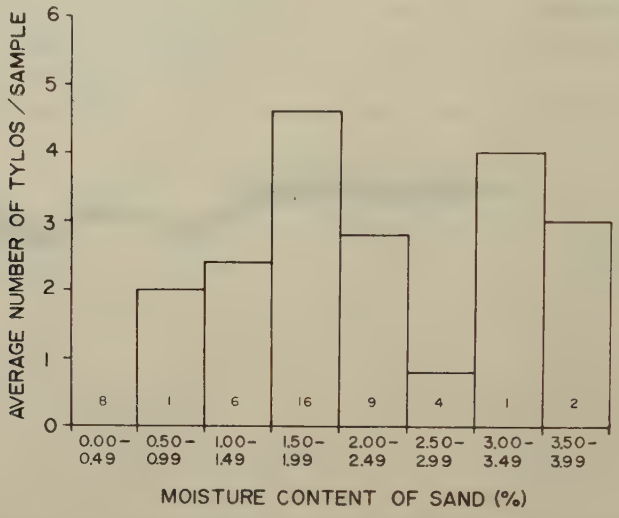


Fig. 4. The average number of animals per core within moisture intervals for the 29 October transect: the number of cores found within each moisture interval is shown within the columns; data from the 0–2 cm cores were not used because of their smaller volu

## Discussion

The sand at Station Beach is made up of a wide range of sizes of shell debris mixed with finely divided quartz with ≈20% of a 50-cm deep core being retained on a 6.4-mm screen and ≈50% on a 3.2-mm screen (Hendrickson, pers. obs.). However, these particles are not well-mixed and occur in random layers of semi-sorted small lenses (<5 cm thick and 50 cm in diameter) with differences in dominant particle size visible to the eye. Analysis of the cores indicate that highly complicated patterns of temperature and especially moisture occur in this beach, and many of these patterns may be the result of the small-scale sorting of sand.

Despite the occurrence of the irregular temperature and moisture patterns, our results suggest *T. punctatus* burrows downward on the beach until it reaches sand with at least 1% moisture. Brusca (1966) reported that *Alloniscus perconvexus*, a central California isopod, also burrows to varying depths in sand beaches according to sand moisture and inhabits sand with 2.9–5.5% moisture content. Our data for *Tylos punctatus* show a peak in numbers on both dates at the 1.5–2.0% moisture range, suggesting a preference for that level. A Kruskal–Wallis analysis of variance of the abundance–moisture data from 29 October indicates that the distribution of

*T. punctatus* with respect to moisture is significantly non-random (probability >0.995). However, the data are limited because of the small sample sizes and the high degree of variability.

Reaching this conclusion was facilitated because the occurrence of relatively constant temperatures on the beach on 29 October enabled us to isolate the effect of moisture from variations in temperature. Because the relationship of animal abundance to moisture and depth remained similar in the two transects while temperature profiles varied markedly, the effect of temperature on burrowing depth was either similar to moisture or had little effect. Laboratory experiments with other oniscoids indicate that given a range of tolerable temperatures, they aggregate in the lower portion of the available range (Warburg, 1964).

Studies of other oniscoids indicate that they seek shelter where the relative humidity is at or near 100% for part of the day since only at this level is moisture loss through the cuticle reduced to zero (Gunn, 1937; Waloff, 1941; Edney, 1968). *Tylos* spp. are structurally similar to their marine ancestors (Vandel, 1943 in Kensley, 1974); therefore, we assume they lose moisture freely through their cuticle in dry air. A preliminary experiment in our laboratory showed that all of 25 animals held in sand with 1.1% moisture at 33 °C for 12 h survived, but that 14 out of 25 died when held at the same temperature but with 0.1% moisture.

We suggest that, while the daytime burrowing of *T. punctatus* serves to remove it from environmental extremes on the open beach and to prevent it being washed out by the high ti (Kensley, 1972), as well as to provide protection from diurnal predators (Hamner *et al.*, 1969), the depth to which the animals burrow is controlled by the need to reach saturation humidities in the surrounding sand.

## Acknowledgements

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# STABLE CARBON ISOTOPE RATIOS IN *FUNDULUS HETEROCLITUS* (L.) MUSCLE TISSUE AND GUT CONTENTS FROM A NORTH CAROLINA *SPARTINA* MARSH[1]

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**Abstract:** Recent food habit and productivity research on the killifish, *Fundulus heteroclitus* (L.), suggests that this species is important in the movement of organic material within and out of the salt marsh ecosystem. The stable carbon isotope ratios of *Fundulus* muscle tissue and gut contents were measured seasonally to determine the carbon sources of the killifish. For muscle $\delta^{13}C$ values ranged from $-13.9‰$ to $-15.8‰$ and $-15.8‰$ to $-18.4‰$ for gut contents, suggesting differential assimilation of ingested material. Muscle tissue values indicate that assimilated carbon probably originated from a mixture of benthic algae and *Spartina* ingested by major prey species: the fiddler crab, *Uca pugnax* (Smith); the polychaete, *Nereis* (*Neanthes*) *succinea* Frey and Leuckart; the tanaid, *Leptochelia rapax* Harger; and other small crustaceans. Small fish (age 0–1 yr) showed slightly lower $\delta^{13}C$ values than larger fish (age 1–3 yr), consistent with differences in their feeding habits. The only seasonal pattern was an enrichment in tissue $^{13}C$ that occurred for both small and large fish in the spring, corresponding to the peak spawning period. The $\delta^{13}C$ determination is a useful tool in identifying and tracing carbon sources through the salt marsh food web, detecting differences in feeding habits between age classes of organisms, and assessing the relative nutritive value of ingested dietary items if differential assimilation is suspected.

## INTRODUCTION

The killifish, *Fundulus heteroclitus*, is an abundant resident of shallow estuarine waters along the east coast of America (Bigelow & Schroeder, 1953) and often follows the rising tide to feed in salt marshes (Butner & Brattstrom, 1960; Kneib & Stiven, 1978). Estimates of *Fundulus* growth and production in marshes are among the highest reported for fish (Valiela *et al.*, 1977; Kneib & Stiven, 1978; Meredith & Lotrich, 1979) and suggest the importance of killifish in the transformation of energy within the estuarine system. *F. heteroclitus* may also affect the distribution and abundance of some marsh invertebrates (Vince *et al.*, 1976), thus implicating killifish as a force structuring the faunal community. Valiela *et al.* (1977) also suggest that the high productivity of this species, coupled with its tidal movements, point to its possible role in

[1] Contribution No. 408 of the University of Georgia Marine Institute.

the export of organic material from the salt marsh. However, before these suggestions can be accepted, the salt marsh carbon sources of the killifish must be clarified, both with regard to original carbon source and intermediate steps in carbon transfer.

An appropriate and relatively uncomplicated method of approaching this problem is by analysis of $^{13}C/^{12}C$ ratios. The marsh flora at the base of the food chain has a wide and distinguishable range of $^{13}C/^{12}C$ ratios depending on its characteristic photosynthetic pathways (Haines & Montague, 1979). The ratios are maintained during passage through the trophic levels (Parker, 1964; DeNiro & Epstein, 1978; Haines & Montague, 1979) and act as natural tracers. The $\delta\,^{13}C$ values obtained by the following formula represent the cumulative effects of carbon assimilation from particular sources:

$$\delta\,^{13}C = \left[ \frac{^{13}C/^{12}C \text{ sample}}{^{13}C/^{12}C \text{ standard (PDB)}} - 1 \right] \times 1000.$$

This has a distinct advantage over gut content analysis which represents an instantaneous view of an organism's diet that may include items not readily digestible and of dubious nutritional value.

In this paper we report $\delta\,^{13}C$ values for *F. heteroclitus* (Linnaeus) muscle tissue and gut contents from a North Carolina salt marsh. These data are compared with dietary data previously collected in the study area and the $\delta\,^{13}C$ values of the principal dietary items of the fish. Since primary prey items vary with size changes in *F. heteroclitus* (Kneib & Stiven, 1978), small (age 0–1 yr) and large (age 1–3 yr) fish are treated separately. We suggest testable hypotheses that would help clarify patterns and seasonal trends in the data.

## Materials and Methods

The study area is a salt marsh surrounding Tar Landing Bay near Beaufort, North Carolina. A detailed description of the study site is given by Kneib & Stiven (1978). The marsh flora is dominated by the cordgrass, *Spartina alterniflora*, giving way to a narrow band (about 5 m) of *Juncus roemarianus* and mixed shrubs fringing the higher edges of the marsh proper.

*Fundulus heteroclitus* were captured in standard wire mesh minnow traps, baited with fresh crushed mussels (*Geukensia demissa*). Bait was placed in fine mesh bags to prevent contamination of the gut contents. Fish were trapped during or immediately following flood tide to allow sufficient time for feeding on the marsh surface. The traps were fished for 30 min either on the marsh surface, in a tidal pool, or in a tidal creek draining the marsh.

Live specimens were taken to the University of North Carolina Institute of Marine Sciences, Morehead City, North Carolina, located 10 min from the study site. Fish were separated into two size groups, corresponding to individuals aged 0–1 yr

(small) and those aged 1–3 yr (large). Usually a sample consisted of at least 10 large and 20 small fish. It was necessary to pool samples within size classes to obtain a sufficient quantity of gut contents for analysis. Total length in millimeters was recorded before each fish was decapitated and the gut separated from the remainder of the body. Specimens were then placed on ice and transported to the University of North Carolina, Chapel Hill, North Carolina where the fish were scaled and filleted before being placed in 10% HCl. Samples were then washed in distilled water and dried at 60 °C for 3–5 days before being ground in a Wiley mill using a No. 40 sieve. Similarly treated were three surface sediment samples taken in April, 1978 from a shallow marsh pool in the study area and samples of marsh invertebrates collected in July, 1979. The invertebrates were held live for 12 h to clear the guts before processing. Only the October sample was not processed immediately upon arrival at Chapel Hill. It was frozen for two months before being treated as above.

Then the ground, dried samples were sent to the third author at the University of Georgia Marine Institute, Sapelo Island, Georgia where the carbon analyses were performed as described in Haines & Montague (1979).

Briefly, samples were combusted in either a Parr bomb or a Phillipson microbomb (depending on the size of the sample). The released $CO_2$ was isolated and collected in sample flasks, and the isotopic ratios determined on a VG Micromass 602 C isotope ratio mass spectrometer in the laboratory of Dr. David Wenner, University of Georgia, Athens, Georgia. Isotope ratios are reported relative to the Chicago PDB standard. The analytical precision of the method is $\pm 0.2‰$ (Haines, 1976), based on replicate determinations of plant material.

## Results

### Isotope ratios of *Fundulus heteroclitus*

Monthly $\delta\,^{13}C$ values for *Fundulus* muscle tissue and gut contents, the number of fish, and the mean total length of each size class used in the determinations are given in Table I. Lengths of sampled fish fluctuated, reflecting the effects of growth, mortality, and recruitment on each size class.

The $\delta\,^{13}C$ values obtained for *F. heteroclitus* muscle tissue ranged from $-13.9‰$ to $-15.8‰$, values intermediate to those reported by Haines & Montague (1979) for *Spartina alterniflora* and benthic algae (Fig. 1). The $\delta\,^{13}C$ values obtained for gut contents $-15.8‰$ to $-18.4‰$ were strikingly lower than those of the muscle tissue. Since the analytical precision of the $\delta\,^{13}C$ technique is $\pm 0.2‰$ (Haines, 1976), we consider the difference between muscle and gut contents as significant, suggesting differential assimilation.

The only seasonal feature in the $\delta\,^{13}C$ values for both size classes of fish muscle tissue was a spring peak that corresponded to the primary spawning period for *Fundulus heteroclitus* in the study site (Kneib, 1976).

TABLE I

Mean lengths (total length in mm) and associated $\delta^{13}C$ values for *Fundulus heteroclitus* in each size class by month.

| Month | Mean length in mm ± SE | *n* | $\delta^{13}C$‰ PDB muscle tissue | Gut contents |
|---|---|---|---|---|
| Jan. | 39.7 ± 1.4 | 11 | −14.9 | Insufficient material |
| Feb. | 62.9 ± 4.8 | 7 | −15.2 | Sample lost |
| Mar. | | | | |
| Large | 72.3 ± 4.4 | 10 | −14.8 | −18.2 |
| Small | 43.9 ± 1.0 | 34 | −15.7 | −15.9 |
| Apr. | | | | |
| Large | 58.9 ± 1.5 | 10 | −13.9 | −15.8 |
| Small | 44.3 ± 0.7 | 20 | −14.2 | (−9.7*) |
| June | | | | |
| Large | 88.0 ± 2.2 | 10 | −15.1 | −16.7 |
| Small | 51.1 ± 0.5 | 25 | −15.8 | −16.5 |
| Aug. | | | | |
| Large | 70.3 ± 1.7 | 10 | −15.2 | Sample lost |
| Small | 45.4 ± 1.3 | 20 | −15.2 | −16.8 |
| Oct. | | | | |
| Large | 81.7 ± 2.6 | 9 | −14.8 | −18.4 |
| Small | 47.6 ± 0.8 | 20 | −14.8 | −16.5 |

* This value is higher than anything reported from the salt marsh. The sample was analyzed separately and may have been contaminated.

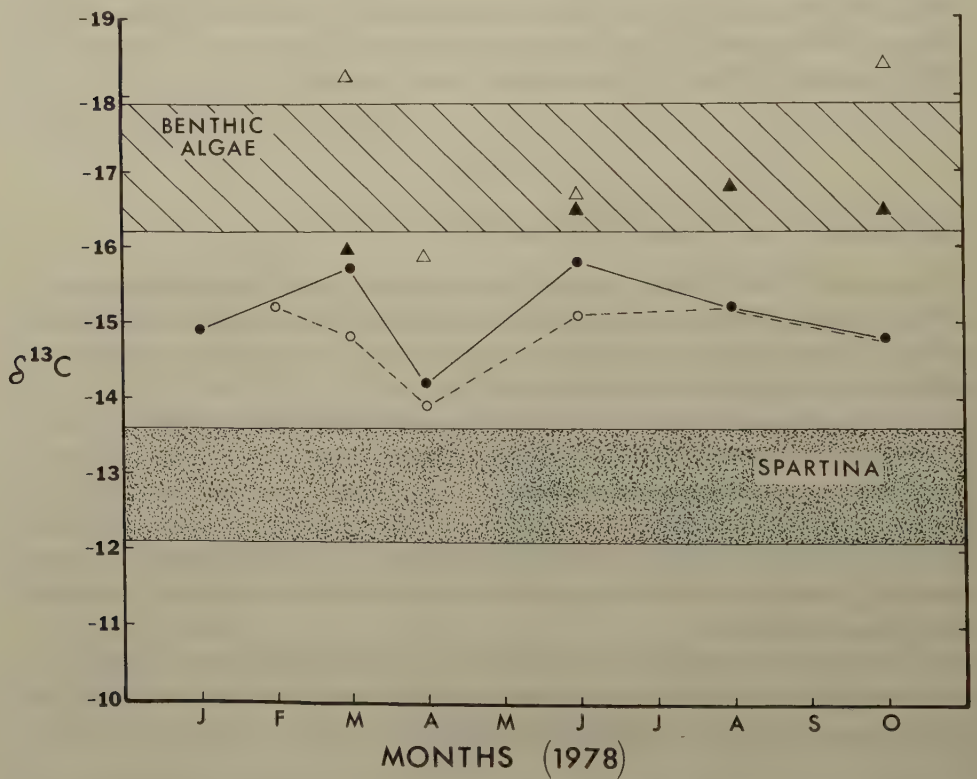


Fig. 1. Seasonal $\delta^{13}C$ values for *Fundulus heteroclitus* muscle tissue and gut contents from a North Carolina salt marsh: ————, small fish; - - - -, large fish; ○, ●, fish muscle tissue; △, ▲, gut contents; shaded areas are ranges for benthic algae and *Spartina alterniflora*; from Haines (1976).

ISOTOPE RATIOS OF *FUNDULUS* PREY

The $\delta^{13}C$ values of common killifish prey organisms are listed in Table II. Values for *Uca* spp. agree with those reported from a Georgia *Spartina* marsh ($-14.4‰$ to $-16.8‰$) by Haines & Montague (1979). The values for the remaining invertebrate

TABLE II

$\delta^{13}C$ values for the principal invertebrates in the diet of *Fundulus heteroclitus*: samples were collected in the Tar Landing study area in July, 1979.

| Prey species | Number of organisms comprising sample | $\delta^{13}C‰$ PDB |
|---|---|---|
| *Nereis succinea* | 10 | −15.8 |
| *Leptochelia rapax* | 350 | −17.3 |
| *Uca pugilator* (adult) | 1 | −16.6 |
| *Uca pugnax* (Smith) (adult) | 8 | −16.4 |
| Immature crabs (3–6 mm carapace width) (*Uca* spp. and *Sesarma* spp.) | 14 | −15.6 |

prey items represent new data. The value for *Nereis succinea* is lower than that reported for another polychaete, *Capitella capitata* (Haines & Montague, 1979). The capitellid, however, was cultured in the laboratory on *Spartina* detritus for three months; its value directly reflects that of its food source.

## DISCUSSION

As a predator, *Fundulus heteroclitus* occupies a top trophic position in the salt marsh food web. We have shown that $\delta^{13}C$ values for the muscle tissue of this species are intermediate to values previously reported by Haines (1976) for benthic algae and *Spartina*. This suggests that the original plant carbon source for the killifish includes components of *Spartina* and benthic algae, assuming that little additional fractionation or differential assimilation has occurred prior to carbon acquisition by the killifish.

Depletion of $^{13}C$ due to fractionation of the source carbon may occur in lipid metabolism (Parker, 1964; DeNiro & Epstein, 1977). If only muscle tissue is used in the analysis (DeNiro & Epstein, 1978, and in the present study), this source of error is avoided.

Numerous $\delta^{13}C$ determinations of animals and their diets from the field have indicated that there is little fractionation of the source carbon as it passes through the food web (Haines, 1976; Fry *et al.*, 1978; Fry & Parker, 1979). Recent laboratory experiments have supported the findings from the field (Haines & Montague, 1979). DeNiro & Epstein (1978) reported small differences (up to $+2‰$) in $\delta^{13}C$ va-

lues of animals compared to their food. $^{13}C$ enrichment in animal tissues compared to the organism's food source has been attributed to the selective loss of the lighter $^{12}CO_2$ in respiration (Fry *et al.*, 1978). McConnaughey & McRoy (1979) have incorporated fractionation effects in a model of the Bering Sea food web with each trophic level (including bacteria and meiofauna) resulting in a 1.5‰ increase in $\delta^{13}C$ values. If we assume this fractionation model for the salt marsh system, an unlikely number of trophic levels would be required to yield the values of *Fundulus* muscle tissue observed in the present study if one starts with either $C_3$ plants ($-24.0$‰ to $-34.0$‰) (Haines, 1976) or phytoplankton ($-20.0$‰ to $-26.3$‰) (Haines & Montague, 1979) as the base carbon source. The phenomenon of $^{13}C$-enrichment through the trophic levels (DeNiro & Epstein, 1978) also casts doubt upon the tenet that *Spartina* is the primary base carbon source. Tissues of both killifish and common marsh invertebrates (Table II) are $^{13}C$-depleted relative to *Spartina*.

Closer examination of the diet of *F. heteroclitus* (Figs. 2, 3) shows that the killifish from our study area feed primarily on polychaetes (notably *Nereis succinea*), small crustaceans (mostly the tanaid, *Leptochelia rapax*), and the fiddler crab (*Uca pugnax*), in addition to some amphipods, isopods, copepods, and ostracods (Kneib & Stiven,

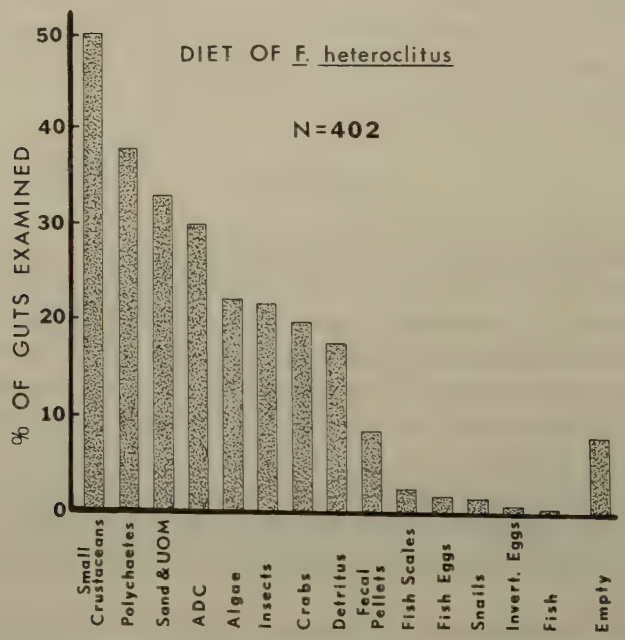


Fig. 2. Percent of *Fundulus heteroclitus* guts containing each of the indicated dietary items (from Kneib, 1976): UOM, unidentified organic material, mostly sediment and bottom debris; ADC, algal–detrital conglomerate, a mixture of living and dead filamentous algae, diatoms, and detritus particles often adhering to *Spartina* stems; both UOM and ADC were used as measures of sediment surface feeding as opposed to feeding in the water column.

1978). Considerable amounts of living algae and bottom debris, including *Spartina* detritus, are also ingested (Fig. 2), especially by larger fish (Kneib, 1976).

Algae, primarily pennate diatoms and flagellates, are abundant on the marsh surface sediments, adhering to *Spartina* stems and detritus particles and accounting for a considerable amount of carbon fixation in the salt marsh system (Pomeroy, 1959; Day *et al.*, 1973). The $\delta^{13}C$ values for the surface sediment samples in our study area ($-17.0‰$ to $-17.8‰$) are typical values for benthic algae. Not surprisingly, the unidentified organic material (UOM) and the algal–detrital conglomerate (ADC) components (see Fig. 2) of the *Fundulus* diet contain large amounts of algae.

Although information on the specific food habits of *Fundulus* prey species is lacking in the literature, most species are generally classified as surface deposit-feeders or grazers, ingesting large quantities of algal and detrital material from the surface sediments (Teal, 1962; Cammen *et al.*, 1978; Fauchald & Jumars, 1979). All sizes of *F. heteroclitus* have a high digestive efficiency for animal material, but most plant material is reported as undigestible (Targett, 1979). Therefore, undigested plant material in the guts of prey species consumed by *Fundulus* probably remains undigested in the fish gut along with other resistant prey components (mandibles and exoskeletons) and ingested plant particles. Jeffries (1972) concluded from fatty acid analysis of *Fundulus* gut contents and muscles that a reasonable diet for this fish included five times as much detritus as marsh invertebrates. Subsequent studies, however, have shown *Spartina* detritus to be of little nutritive value to *Fundulus* (Prinslow *et al.*, 1974).

If *Fundulus* were unable to assimilate much of the material present in the gut, differences in $\delta^{13}C$ values of the gut contents and muscle tissue would be expected. The surprising feature of the gut content pattern is that the values lie consistently in the range of benthic algae. The lack of variation is surprising for an omnivore, although variation between individuals was eliminated by pooling gut contents of all specimens within each size class to obtain sufficient material for analysis.

The two prey items comprising the greatest biomass consumed, especially by larger fish, are *Nereis succinea* and *Uca pugnax* (Fig. 3). Although we found that *Nereis succinea* guts always contain large amounts of detritus, the organism seems to derive an almost equal amount of carbon from algae, the detrital fraction assimilated in major part from the microflora associated with the detrital particles (Cammen *et al.*, 1978). Given its feeding habits and assimilation capabilities, a $\delta^{13}C$ value intermediate to *Spartina* and benthic algae would be expected for *Nereis succinea*.

The tanaids which comprise a larger portion of the diet of smaller *Fundulus heteroclitus* (Kneib & Stiven, 1978) feed primarily on benthic algae (Kneib, personal observation) as reflected in the $\delta^{13}C$ value for *Leptochelia rapax* (Table II). This would be expected to cause an appropriate shift in $\delta^{13}C$ values for fish flesh and could account for the small, but consistent, difference in the flesh values for large and small fish.

The $\delta^{13}C$ enrichment in *Fundulus* muscle that was observed in April may be related

to the spring spawning peak of the species. The eggs of *Fundulus* contain fatty deposits (Marza *et al.*, 1937) forming vacuoles that concentrate at one pole in the ripe ova. If lipids are drawn from other somatic tissues to satisfy the lipid demand in egg production, the result would be to increase the $\delta\ ^{13}C$ value for the tissue depleted in lipids since lipids are depleted in the $^{13}C$ isotope. This hypothesis is easily testable. If lipids are drawn from muscle tissue to supply eggs, $\delta\ ^{13}C$ values of female muscle tissue should be higher than that of males during the major spring spawning period. In the present study the sexes were not analyzed separately.

An alternative hypothesis would be that the increase in flesh $\delta\ ^{13}C$ was in response to an acute change in diet. A prerequisite for this explanation is a very rapid carbon turnover in the muscle tissue of *F. heteroclitus*. Sufficient carbon turnover to pro-

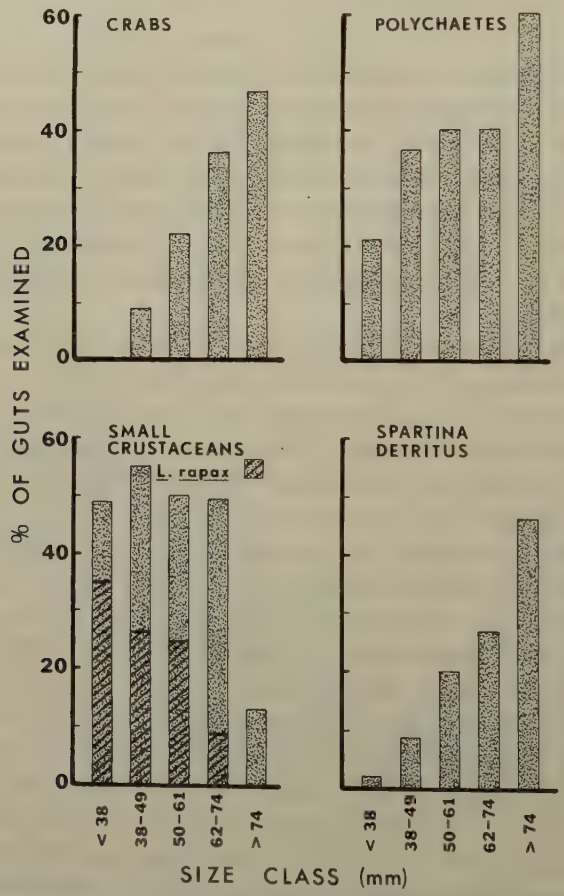


Fig. 3. Percent of *Fundulus heteroclitus* guts containing selected dietary items presented for five size classes of fish: the number of fish examined in each size class were from smallest to largest: 47, 111, 152, 77, and 15, respectively (from Kneib, 1976); sizes are total length in mm; crabs were primarily *Uca pugnax*; polychaetes were primarily *Nereis succinea*; small crustaceans were primarily *Leptochelia rapax*, as indicated.

duce the observed $\delta\,^{13}C$ change in only one month is unlikely (Haines & Montague, 1979).

In conclusion, the $\delta\,^{13}C$ determinations confirmed the dietary analysis of *F. heteroclitus* carried out by the more traditional technique of gut content analysis; in addition, they point out the possibility of differential assimilation of ingested material. Our data also indicate that the significance of benthic algal production in the salt marsh food web may have been underestimated in the past, overshadowed by intensive study of the more conspicuous floral component, *Spartina alterniflora*, the influence of which may be more as a structural component (Bell *et al.*, 1978) of the community than as the primary trophic component.

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# DIFFERENTIAL SUSCEPTIBILITY TO GRAZERS IN TWO PHASES OF AN INTERTIDAL ALGA: ADVANTAGES OF HETEROMORPHIC GENERATIONS

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**Abstract:** The intertidal red alga *Gigartina papillata* (C. Ag.) J. Ag. alternates between two major forms in its life cycle, an upright blade and a fleshy crust. The evolution and functional significance of such alternation of heteromorphic generations is little understood. This study, done on the outer coast of Washington state (U.S.A.), considers the rôle of physical factors, measured by intertidal position, and a biological factor, interactions with herbivores, as selective pressures potentially responsible for this pattern. Observations along transects and a herbivore exclusion experiment showed that the two phases of this alga simultaneously occupy similar intertidal positions but differ significantly and oppositely in their response to grazing. The number of crust plants declined in the absence of grazers, probably due to being overgrown and shaded by a thick algal film. It is, therefore, suggested that the crust form is grazer-dependent. In contrast, the number of blade phase plants increased in the absence of grazers. "Bet-hedging" is suggested in explanation of the maintenance of two phases in one cycle; the crust is more persistent but susceptible to overgrowth, while the more productive blade is more susceptible to grazing.

## INTRODUCTION

The intertidal red alga *Gigartina papillata* has a heteromorphic life cycle, alternating between a fleshy (non-calcified) crust and an upright blade (West, 1972; Polanshek, 1975). The adaptive significance of such heteromorphic, diplobiontic life cycles (Bold & Wynne, 1977) is not clearly understood. The maintenance of heteromorphic phases in one life cycle suggests that each form has advantages, and that neither one can predictably succeed in all circumstances. We can ask, what are the relative advantages of each growth form, and what factors in selection create and maintain that advantage? This type of cycle allows an examination of form and its implications within one life cycle, eliminating problems involved with the comparison of different morphologies between species.

Algal form has been seen to vary as a function of physical factors such as wave exposure and light (Widdowson, 1965; Druehl, 1968; Palmisano & Sheng, 1977). Experimentally varying these factors can influence changes in form (Sundene, 1964; Kim, 1976; Steneck & Adey, 1976; Bonneau, 1977). These results imply that shape

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is an adaptation to the physical environment, and that different shapes would be expected to be found in different locations. Biological factors, such as herbivores, are also known to be important in the distribution and abundance, seasonal variation, species composition, colonization, and succession of benthic algae (reviewed in Southward, 1964; Jones & Kain, 1967; Paine & Vadas, 1969; Dayton, 1971; Menge, 1975; Lubchenco, 1978). Morphology may be expected to be important in these kinds of interactions, especially for two such different shapes as a crust and blade. This study examines the relative importance of physical processes and grazing on the survival of the two markedly different morphological phases of *G. papillata* (C. Ag) J. Ag. over 11 months (Sept. 1976 to Aug. 1977). Two hypotheses were tested: (1) if the heteromorphic phases of this plant have different physiological and mechanical tolerances, they would be expected to occur in different positions in the intertidal zone, and (2) if the life-history phases are differentially susceptible to grazing, they would be expected to respond differently to experimental decreases in grazer density. Overlapping intertidal position of the crust and blade phase would suggest that physical factors correlated with intertidal height are not the primary determinants of the two phases. Differential responses to herbivore removal would, however, support the second hypothesis.

## Site Studied and Organisms

The field site was located on the protected outer coast (after Ricketts *et al.*, 1968) on Waadah Island in the Strait of Juan de Fuca, located on the Olympic penin-

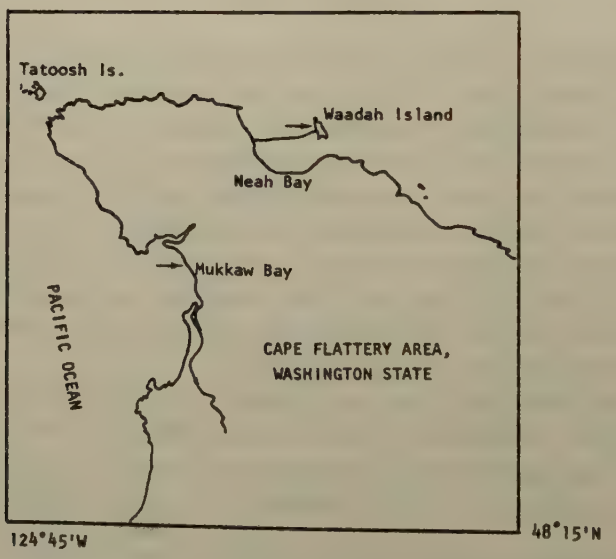


Fig. 1. Location of the area in studied Washington State: the primary site is Waadah Island.

sula of Washington state (48°19′N : 124°44′W) U.S.A. (Fig. 1). The area studied was a vertical wall of sedimentary rock at +1.8–2.5 m above mean low low water (M.L.L.W.) (see Paine *et al.*, 1979). The area is free of human disturbance (access is limited by the U.S. Coast Guard).

The most abundant macroalga on the wall is the *G. papillata* complex. West (1972) showed that the prostrate fleshy crust (commonly called "tar spot"), formerly *Petrocelis franciscana* Setch. et Gardn., now *P. middenforffii* (Ruprecht) Kjellman (Polanshek & West, 1975) produces tetrapores which grow in culture into an upright thallus morphologically similar to *Gigartina agardhii* Setch. et Gardn. Polanshek (1975) established the existence of a similar cycle for *G. papillata*. Hinchman (1964) placed both these intertidal species on the Washington coast in the *G. papillata* complex but West *et al.* (1978) have recently separated them on the basis of culture work. Kim (1976) proposed the transfer of some heteromorphic species of *Gigartina* into the subgenus *Mastocarpus*. In the present paper Hinchman's usage is followed, and descriptive names for the separate phases are used, e.g. crust for the tetrasporophyte phase formerly known as *Petrocelis*, and blade for the gametophyte phase formerly called *Gigartina*.

Other large algae in the area studied include: the fleshy crust *Hildenbrandia occidentalis* Setch.; the coralline crust *Lithophyllum* sp.; and two ephemeral blades (winter and spring only), *Porphyra pseudolanceolata* Krishnam. and *P. abbottae* Krishnam. The principal grazers at the site are the acmaeid limpet *Collisella digitalis* Ruthke, and the snails *Littorina sitkana* Philippi and *L. scutulata* Gould. The wall is bare of other animals except the barnacles *Balanus glandula* Darwin and *Chthamalus dalli* Pilsbry.

## Methods

### TRANSECTS

Four transects were sampled at Waadah Island, and three at Mukkaw Bay (Fig. 1) to compare the distribution of the crust and blade phases of *Gigartina* in late spring, 1976. These transects were laid out perpendicular to the water line and varied from 37–98 m in length. Sampling was continuous along the transect line (using a 0.25 $m^2$ quadrat) which extended from the waterline at low tide (−0.8 m, above M.L.L.W.) to a point above the range of *Gigartina*. These data were recorded as simple presence or absence of each phase, as the relative abundance varied so little (0–2% cover by holdfast or thallus in blade or crust, respectively).

### HERBIVORE EXCLUSION

To test the effects of grazing on the relative survival of the two phases, the wall studied was arbitrarily divided into 10 strips; herbivores were counted and removed from five strips and five were left undisturbed as controls. Each strip was 45 cm

wide and at least 1.5 m long, and was further marked into three 45 × 45 cm areas – low (L), medium (M), and high (H) (Fig. 2). Strips were marked with copper based anti-fouling paint to decrease the migration of grazers (as used by R. Paine and E. Nicotri, pers. comm., and Cubit in Carefoot, 1977). The paint was renewed when it showed signs of wear. A fence of Vexar (TM Dupont) plastic mesh, anchored with screws and bonded to the rock with marine cement was built at the base of the site as a barrier to herbivore migration into it. The number and position of grazers was recorded at roughly monthly intervals on both the control and test strips; those on the test strips were moved outside the area being studied.

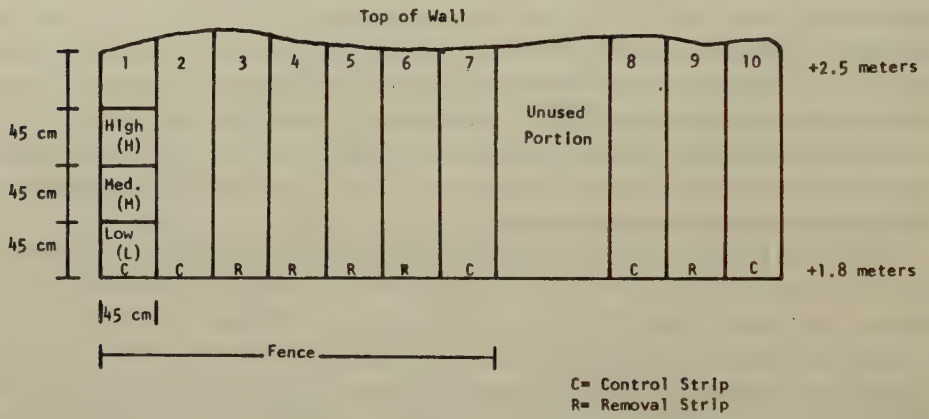


Fig. 2. Drawing of the wall studied, showing design of herbivore exclusion experiment.

Changes in algal abundance were assessed by maps made at the beginning, middle and end of the experiment. Major topographical features, such as cracks and holes in the wall were noted, to identify blade and crust plants. Blade plants were counted individually; crust plants occurred in discrete, identifiable circles.

## Results

### DISTRIBUTION PATTERNS: ALGAE

Data from the seven transects show that the crust and blade phases of *Gigartina* co-occur in space and time throughout most of the intertidal zone, and that both phases occupy similar positions within it, barring micro-habitat differences (Fig. 3). Thus the two phases do not appear to have different tolerances as reflected in physical factors correlated with tidal height, such as desiccation. In the limited area of the study wall alone, both crust and blade phase occur at all heights (H, M, and L, Fig. 2), but the center of the crust population is higher than that of the blade (Table I).

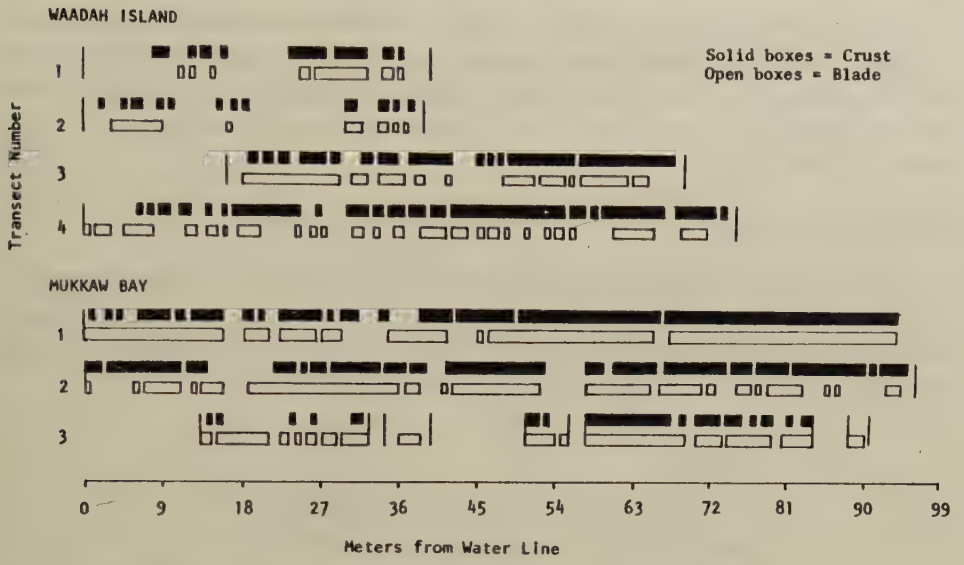


Fig. 3. Distribution patterns of crust and blade phases of *Gigartina papillata*: transects from low to high intertidal zone.

TABLE I

Abundance (no./0.2 $m^2$) of crust and blade phase plants and individuals of the three grazer species relative to tidal height on the study area wall prior to initiation of the experiment (September 1976).

| | Crust | Blade | *Collisella* | *L. sitkana* | *L. scutulata* |
|---|---|---|---|---|---|
| High | 14 | 1 | 405 | 116 | 120 |
| Medium | 43 | 3 | 216 | 133 | 213 |
| Low | 42 | 53 | 24 | 48 | 206 |

## GRAZERS

Before any manipulation was begun, the three herbivores to be considered were found in different abundance at different tidal heights on the study wall (Table I). *Collisella digitalis* and *Littorina sitkana* were more abundant in high and medium portions, and less abundant below, while *L. scutulata* was more abundant in medium and low portions and less abundant above. The number of all grazers was not significantly correlated with crust plants, but was negatively correlated with numbers of blade plants (Spearman rank correlation test, Siegel, 1956, $P \leqslant 0.05$). When each species of grazer was compared separately with each of the two algal phases, a negative correlation was seen between the blade and the limpet, and a positive correlation between crusts and *L. scutulata* ($P \leqslant 0.05$)). When strips and heights were treated as separate quadrats negative correlations were seen between the following pairs: crusts and *Collisella*, blades and *Collisella*, and crusts and *Lit-*

*torina sitkana*. Thus, there is often an inverse correlation between the abundances of limpets and blades, and in larger sample treatments the abundance of crusts may also be negatively correlated with limpet numbers. Further analysis suggests, however, that this correlation is due to the response of grazers and algae to a third factor viz, tidal height.

## HERBIVORE EXCLUSION

Exclusion of the limpet from the test strips was very effective, with a clear difference between numbers of grazers in the control and test strips (Fig. 4); this grazer was presumed important on the basis of correlations. Densities of littorine snails were decreased (Figs. 5 and 6), especially *L. scutulata* (Fig. 6).

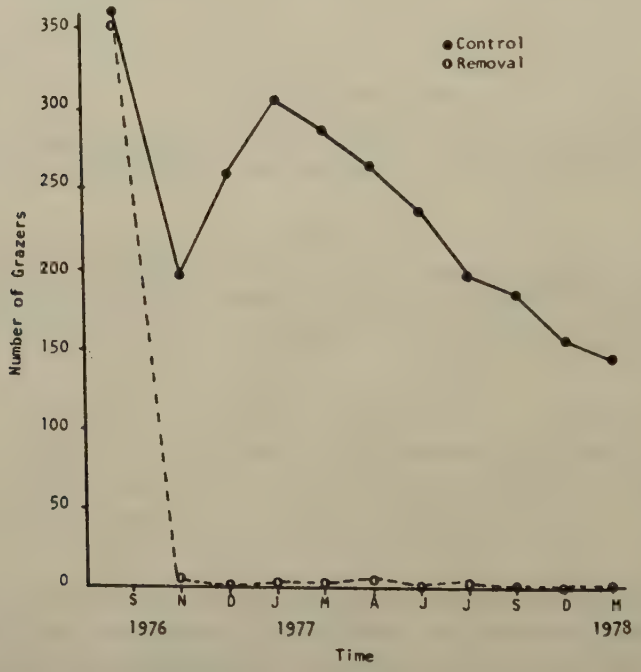


Fig. 4. Effectiveness of exclusion for *Collisella digitalis*.

The effects of the herbivore exclusion experiment were assessed as changes in numbers of crust and blade phase plants in control versus test strips, and changes in the abundance of other organisms. One month after the experiment was begun, a thick algal film or mat was visible on all the test strips, but not on any controls. The film was several mm thick, composed of diatoms, green, brown, and red algae of various forms and stages of development, and the tube-dwelling chironomid midge larvae (*Paraclunio alaskensis* Coquillet). The mat first appeared in the fall,

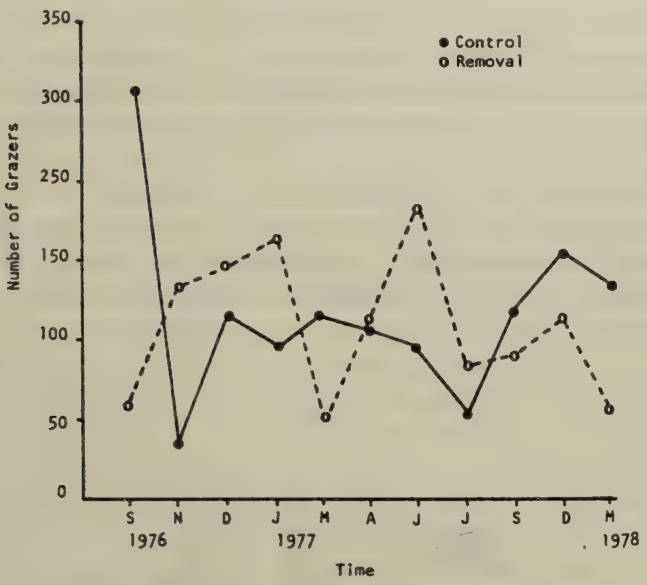


Fig. 5. Effectiveness of exclusion for *Littorina sitkana*.

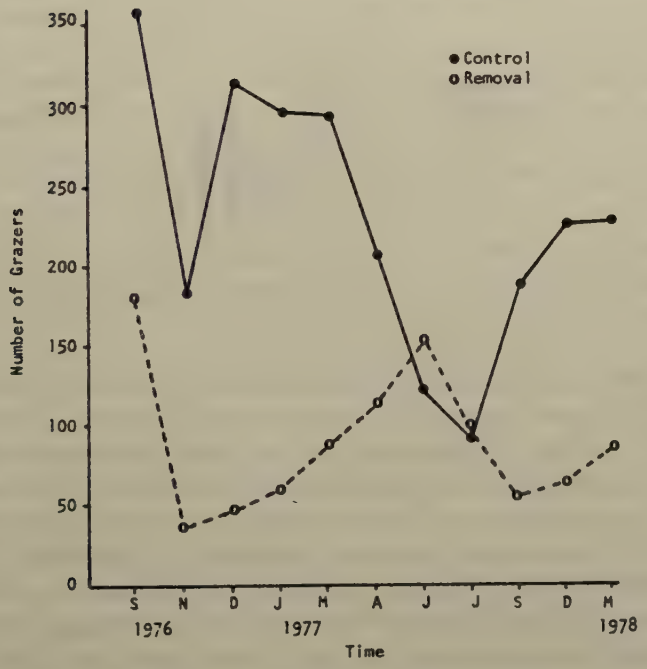


Fig. 6. Effectiveness of exclusion for *Littorina scutulata*.

remained throughout the winter, and decreased to a thinner layer in late spring and summer. In addition, *Porphyra pseudolanceolata*, an ephemeral red blade, covered the higher portions of all the test strips in winter and spring. At the maximum thickness of this film it was difficult or impossible to locate the boundaries of the crust plants.

The numbers of crust and blade phase plants in the control and test strips were similar at the beginning of the experiment (Mann–Whitney $U$-test, Sokol & Rohlf, 1969, $P > 0.05$) but not at the end. Where herbivores had been excluded or decreased, significantly more blades became established and crusts disappeared, relative to control areas (Fig. 7; Fisher's Exact test, Zar, 1974, $P \leq 0.05$)). The

Fig. 7. Changes in numbers of crust and blade phase plants after exclusion of grazers.

data were plotted as follows: sign was determined by following an individual plant from autumn 1976 to autumn 1977. Plants persisting through the year are assigned a zero, those appearing in this period a plus, and those disappearing a minus (horizontal axis, Fig. 7). Fig. 7 shows the differential response of the two life-history phases to grazing. Crust plants showed significantly more minuses (disappearances) in the test than in the control strips, indicating a decrease in the number of plants in the absence of grazers. In contrast, the number of blades increased significantly in the test strips, suggesting that the blade is more susceptible to grazing ($P > 0.01$). Thus herbivores reduce recruitment and survival of blades, while increasing the survival of crusts.

## Discussion

The variety seen in algal form and life cycles has often been dismissed as of accidental or neutral evolutionary significance. The main point of this study is that this variety, specifically within heteromorphic forms in one life cycle, may instead be adaptive.

The existence of a specialized morphology is often interpreted as an adaptation to a particular set of physical conditions such as the tough "leathery" macroalgae living on exposed shores, or the fragile blades living in dim, protected underhangs. Because the most obvious differences in *Gigartina*'s phases are morphological, they might thus be expected to be adaptations to different physical regimes. For example, if the crust phase were more tolerant of desiccation than the blade, it would be expected to be higher in the intertidal than the blade. Or, if the crust has lower light requirements (when submerged) it might extend lower in its range than could the blade. One would expect to see different local distribution patterns of the life history phases if they were adaptations to different physical conditions which vary with tidal height. Since the crust and blade phases of *Gigartina* co-occur throughout the range sampled (Fig. 3), they are exposed to similar physical factors. These transect data (and those of Åsen, 1976) do not support the view that the heteromorphic phases are adaptations to different physical factors.

Fitness is a cumulative measure of an organism's response to various mortality sources in its selective regime. The incorporation of very different morphological phases within one life cycle could be seen as adaptation to different sources of mortality within this regime. For example, the crust form may be resistant to detachment from the substratum, but more susceptible to being overgrown; whereas the blade form may be more productive, but more vulnerable to herbivores. This may be best described as "bet-hedging" (Cohen, 1966) or cost-benefiting. In this study the crust and blade phases differed significantly in response to herbivores (Fig. 7). The number of crust plants decreased in the absence of grazers, probably due to the negative effects of overgrowth by epiphytes. I suggest that the crust phase, itself grazed, is actually grazer-dependent. Grazing which removes epiphytes can be advantageous to the "host" plant (Adey, 1973; Black, 1974; Steneck, 1977; Wanders, 1977; Lubchenco, 1978) especially if the "cost" (loss of tissue) can be minimized, as with a tough, adherent crust (e.g. *Petrocelis*). This type of interaction may be a mechanism in the evolution and maintenance of this and other heteromorphic cycles (see also Slocum, 1978; Lubchenco & Cubit, pers. comm.).

The number of blade plants increased where grazers were removed, either through preferential consumption by grazers (as indicated by experiments with related grazers in California; C. Kitting, pers. comm.) or simple detachment by them from the substratum. The differential susceptibility of the two phases of *Gigartina* to both direct and indirect herbivore effect – grazing and overgrowth – shows the importance of this biological factor in a relatively short time; the rôle of grazers as one

of the agents of selection in the maintenance of heteromorphy is implied.

Mechanisms for interactions of *Gigartina* with grazers are suggested with a brief description of the characteristics of crust and blade phase. Each of the phases has some attributes considered advantageous in certain conditions; alternating the phases in one life cycle includes both sets of advantages, which may be expected to balance disadvantages in such a way as to leave more descendants in the next generation. That both phases are retained in the cycle (rather than one phase) may be inferred to indicate the value of a two-edged strategy. The crust phase is tightly attached to the substratum along approximately half its surface. This mode makes it accessible to grazers but much harder for them to remove. The prostrate forms in plants, as well as sessile invertebrates, are more susceptible to being both shaded and overgrown (Jackson, 1979). Some potential photosynthetic surface area is made unavailable by this mode of attachment. The crust reproduces only once a year (West, 1972; L. A. Hanic, pers. comm.); it grows very slowly but persists throughout the year and is long-lived (some calculated individual ages exceed 40 yr; Paine *et al.*, 1979).

In contrast, the blade phase is attached to the substratum by a narrow, relatively fragile strip of tissue; this renders the plant extremely vulnerable to complete detachment by grazers and subsequent death. Its upright form makes it less likely to be overgrown; a relatively large surface area for photosynthesis may explain its rapid growth rate and frequent reproduction (Hinchman, 1964). The blade phase is, however, ephemeral, with most plants fragmenting in the fall and reappearing in the spring (Hinchman, 1964).

Implicit in the advantage of possessing different phases is the assumption that they are best used in different conditions, the appropriate set of tactics being used in the circumstances in which they are likely to be successful (Stearns, 1966; Istock, 1967; Harper, 1977). In a predictable environment the phases may exist in a kind of dynamic stasis, generations alternating with one another in a life cycle, while both phases coexist in space and time. Within this rhythm however, a modification of tactics in response to the current environmental conditions may bring additional advantage. *Gigartina* shows the ability to alter phase, or tactics, though the cues are not known (see also Wynne, 1969, for similar patterns in brown algae). In the laboratory, the blade phase of *Gigartina* sometimes produces propagules which do not develop into the usual alternate phase crusts, but rather, more blade plants (West & Polanshek, 1972; Polanshek & West, 1975). Non-obligate alternation of phases is seen in other, isomorphic algae, but is especially significant in heteromorphic algae: it suggests that the morphological phase most appropriate to some situation may be used. Possessing heteromorphic phases and some control over their expression, may increase fitness, both in each phase and in the complete cycle of the individual plant.

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# *CONCHOCELIS* DISTRIBUTION IN THE FIRTH OF CLYDE: ESTIMATES OF THE LOWER LIMITS OF THE PHOTIC ZONE

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**Abstract:** A series of transects in the Firth of Clyde have shown that filaments of *Conchocelis* in shell substrata are always the deepest growing red algae. The abundance of calcareous material on the sea bed and its invariable association with the perennial *Conchocelis* filaments offers a reliable means of estimating photic limits. Some interactions of grazing molluscs with the shell-boring organism have been observed. Some of the pitfalls to be encountered in field determinations of *Conchocelis* and its consequent use as an indicator organism are described.

## Introduction

The underwater light climate is a crucial factor governing the growth rates and life history sequences of sublittoral marine algae. Studies by Jerlov (1951) have shown that submarine light at depths of 32 m varies between 10 and 0.05% of the incident surface radiation in a number of types of ocean water and with varying angles of incidence of sunlight. The depth with 1% of the incident surface radiation is often regarded as the lower limit of the photic zone. The interrelationships between the pigment arrays of marine algae and submarine illumination have been extensively studied. It is well known that some marine algae grow successfully in brightly illuminated shore habitats whilst others grow in the dim light of shaded intertidal habitats and the sublittoral. The plants would seem to be adapted to both life in varying ranges of light intensities and to different spectral qualities. The pigment arrays enable the plants to utilize the full ranges of spectral compositions associated with shaded habitats and underwater illumination. Red algae tend to be more abundant in shaded intertidal habitats and in the sublittoral (see reviews in Levring *et al.*, 1969; Hellebust, 1970). Quality of light is not, however, the sole governing factor in seaweed distribution.

*Conchocelis rosea* was first described by Batters (1892) in mollusc shells dredged from the sea bed in the Firth of Clyde. The link between *Porphyra* spp. and the shell-boring *Conchocelis* filaments was first established in the culture studies of Drew (1949, 1954). Similar shell-boring filaments have been recognized as life history

phases of *Porphyra* and *Bangia* spp. worldwide. Drew (1954) also demonstrated that any calcareous material of living or non-living origin would function as substrata for *Conchocelis* growth. *Conchocelis* filaments in mollusc shell fragments were observed by Drew in dredgings taken at 32 m depth, which would be close to the compensation point in some ocean waters according to Jerlov's (1951) data. Sheath *et al.* (1977) have shown that the *Conchocelis* of *Porphyra leucosticta* has a very low compensation point (15 $\mu$W cm$^{-2}$) at 20 °C and that it adapts to dim light conditions by lowered respiratory rates. If no growth takes place the filaments probably remain in a "steady-state" situation, just maintaining themselves. Following dark-storage there is a slow loss of photosynthetic activity on re-illumination, falling in 3 wk to 50% of that of light grown control plants. When kept below their compensation points or in the dark, fragmentation and dilation of the chloroplast thylakoids is observed with formation of tubular units. Whilst the quantity of chlorophyll almost doubles, the phycoerythrin and carotenoid contents remain unchanged. Re-organization of the dark-changed thylakoids is achieved within 24 h of return to light incubation. There is thus clear experimental evidence that the *Conchocelis* of one *Porphyra* species could survive prolonged periods in very dim submarine light. It has been known for some time that seasonal growth periods of *Conchocelis* are in the long days of summer months (Kurogi, 1959; Iwasaki, 1961; Kurogi & Sato, 1962; Kurogi *et al.*, 1962; Iwasaki & Matsudaira, 1963).

Measurements of radiant energy levels with increasing depth are not to be regarded as the sole means of determining photic limits since growth of benthic algae is a long term expression of the plant's response to underwater light regimes which may vary well above or below the 1% surface illumination value with both season and sea condition. The use of macroalgae may enable estimates to be made of the photic limits for a particular area by inference from general floristic work. A more positive indication of such limits would be particularly valuable where there are turbidity gradients, such as those at river mouths or in localities where industrialization may lead to changes in substrata or to increased silt loading of the water. The easy recognition of these limits using macrophytic algae presents problems, notably because of the lack of data through disjunct distributions – possibly due either to herbivore pressures or the absence of suitable substrata. From earlier distributional information and from the experimental data quoted, *Conchocelis* seems well adapted for survival and growth at about the photic limit in subtidal habitats. In an attempt to obtain information on the photic limits in the Firth of Clyde (Clokie *et al.*, 1979), and with reference to the continuing industrialization of the Hunterston shoreline, a series of transects was made and the shell flora studied at various depths.

## Materials and Methods

Two methods of sampling were used. At shallow sites samples were collected by diving. In deeper water collections were made with a Van Veen Grab (Holme &

McIntyre, 1971). The appropriate limits were first bracketed by widely set samples, then collections were made more precisely across the distribution boundaries. With the steep sided nature of underwater terrain in parts of the Firth of Clyde, the positions of closely set sampling stations were often determined by the practical requirements of boat handling. After washing through a 4-mm sieve, any calcareous material obtained was examined. Over most of the depth range *Conchocelis* was easily identifiable. At the deeper stations, confirmation of the presence of *Conchocelis* required decalcification using Lillie's fixative (50 g picric acid dissolved in 850 ml sea water with 100 ml of 40% formaldehyde and 50 ml formic acid then added). If required, permanent slides were then prepared in Canada Balsam after dehydration in an alcohol series and final clearance in xylol. By assessing the presence or absence of a species at two different but close sampling points, it was possible to establish a graphical summary of species reduction with increasing depth. The benthic foliose and filamentous marine algae in the Firth of Clyde grow down to about one-third of the depth reached by the deepest non-superficial shell-boring algae. The photic limits were estimated by interpolation between the deepest positive occurrence and the shallowest negative for *Conchocelis*. The reliability of the sampling method was stretched at some sites due to the irregular nature of sea bed which prevented the photic limits, as defined by the presence of *Conchocelis*, being fixed within narrow limits. The *Conchocelis* lower limit is hence defined as the mean depth between the deepest positive record and the shallowest negative for any sampling station, and expressed as the depth in metres.

*Conchocelis* is here used as a "form class", since to link the shell-boring phase with *Porphyra* spp. and *Bangia atropurpurea* (Roth.) C.Ag. would require culture experiments. Nevertheless its use as a "form class" gave every indication of yielding consistent and reliable data. With full knowledge of the significance of the associated organisms and of shell colours a robust sampling technique became available.

Material required for Stereoscan observations was fixed in formalin, dehydrated through a series of alcohol concentrations and then critical point dried from ethyl acetate.

## Results

The details of a transect measured on a west-facing sublittoral cliff-face off Little Cumbrae are given in Table I and summarized graphically in Fig. 1. Halfway to the photic limit (based on *Conchocelis*), with the exception of *Lithothamnium sonderi* Hauck., all of the other species were absent. If solid substrata (pebbles) are present, then *L. sonderi* may be found as deep as *Conchocelis*, but the inevitable presence of shell fragments no matter what the nature of the sea bed means that *Conchocelis* is invariably found. The species reduction curve (Fig. 1) shows the lengthy depth prolongation due to *Lithothamnium sonderi* and *Conchocelis* and the noticeable decline in species between 10–12 m. The apparent increase in species numbers below

the sublittoral fringe (2–3 m) may well be due to a sampling artefact. Noticeably the depth distribution of some species above 10–12 m is of a much wider range than others, as may be seen by comparing *Phycodrys rubens* (which also shows accompanying morphological changes) and *Sphacelaria brittanica*. The method of determining the lower limit of *Conchocelis* is shown in Fig. 2, in which the deepest positive occurrence and the shallowest negative are shown. The chiton *Lepidopleurus asellus* Gmelin is a characteristic component of the deep water fauna in the region of the photic limit as defined by *Conchocelis*.

TABLE I

Sublittoral algal distribution on transect off Little Cumbrae, June 1976: depths measured from Chart Datum; upper limit shown as * where in sublittoral fringe or eulittoral, limits within sublittoral shown as depths (m); for taxonomic authorities see Parke & Dixon (1976).

| Species | Upper limit | Lower limit | Species | Upper limit | Lower limit |
|---|---|---|---|---|---|
| *Acrosiphonia arcta* | * | 0.6 | *Callophyllis laciniata* | 9.0 | 11.0 |
| *A. aeruginosa* | * | 0.6 | *Ceramium rubrum* | * | 5.0 |
| *Cladophora rupestris* | * | 0.6 | *C. flabelligerum* | * | 4.0 |
| *Monostroma fuscum* | * | 9.0 | *Cryptopleura ramosum* | 2.0 | 4.0 |
| *Phaeophila viridis* | * | 0.6 | *Delesseria sanguinea* | * | 10.0 |
| *Ulva lactuca* | * | 0.6 | *Erythrotrichia carnea* | * | 6.0 |
| *Desmarestia aculeata* | * | 7.0 | *Erythrotrichia* sp. | 7.0 | 9.0 |
| *D. viridis* | * | 3.4 | *Gigartina stellata* | * | 0.9 |
| *Ectocarpus fasciculatus* | * | 7.0 | *Heterosiphonia plumosa* | * | 0.9 |
| *E. siliculosus* | * | 9.0 | *Nitophyllum punctatum* | 1.6 | 6.0 |
| *Fucus serratus* | * | 0.6 | *Odonthalia dentata* | * | 0.9 |
| *Giffordia hinksiae* | * | 4.6 | *Palmaria palmata* | * | 7.0 |
| *G. granulosa* | * | 0.7 | *Phycodrys rubens* | * | 13.4 |
| *Giffordia* sp. | * | 0.8 | *Polysiphonia elongata* | * | 6.0 |
| *Isthmoplea sphaerophora* | * | 4.6 | *P. urceolata* | * | 9.0 |
| *Laminaria digitata* | * | 2.6 | *Ptilota plumosa* | 4.0 | 5.0 |
| *L. hyperborea* | * | 1.4 | *Porphyropsis coccinea* | 4.0 | 7.0 |
| *L. saccharina* | * | 6.6 | *Rhodomela confervoides* | * | 0.8 |
| *Litosiphon laminariae* | * | 6.6 | *Ahnfeltia plicata* | * | 0.8 |
| *Myrionema strangulans* | * | 0.8 | *Cruoria pellita* | 0.9 | 11.0 |
| *Saccorhiza polyschides* | 4.0 | 5.0 | *C. rosea* | 6.9 | 9.0 |
| *Sphacelaria brittanica* | 7.0 | 11.0 | *Dumontia filliformis* | * | 9.0 |
| *S. fusca* | 3.0 | 6.6 | *Fosliella farinosa* | 9.0 | 11.0 |
| "*Aglaozonia*" stage | 1.8 | 3.8 | *Halarachnion ligulatum* | 7.0 | 8.8 |
| *Pseudolithoderma extensum* | 2.8 | 11.0 | *Hildenbrandia prototypus* | * | 0.9 |
| *Antithamnion floccosum* | * | 2.7 | *Litophyllum incrustans* | * | 0.9 |
| *A. spirographidis* | 5.0 | 9.0 | *Lithothamnium glaciale* | * | 13.6 |
| *Audouinella caespitosa* | 9.4 | 10.6 | *L. sonderi* | 8.4 | 27.0 |
| *A. purpurea* | * | 4.8 | *Petrocelis cruenta* | * | 1.0 |
| *A. secundata* | * | 7.0 | *Phymatolithon laevigatum* | 2.0 | 10.0 |
| *A. virgatula* | * | 0.8 | *P. lenormandii* | * | 2.0 |
| *Brongniartella byssoides* | 5.0 | 7.0 | *P. polymorphum* | * | 3.0 |
| *Callithamnion byssoides* | 7.0 | 11.0 | *Rhodophysema elegans* | 7.0 | 9.0 |
| *C. corymbosum* | 9.0 | 11.0 | "*Trailliella*" stage | 2.0 | 11.0 |
| *C. hookeri* | * | 0.8 | *Conchocelis* | * | 27.0 |

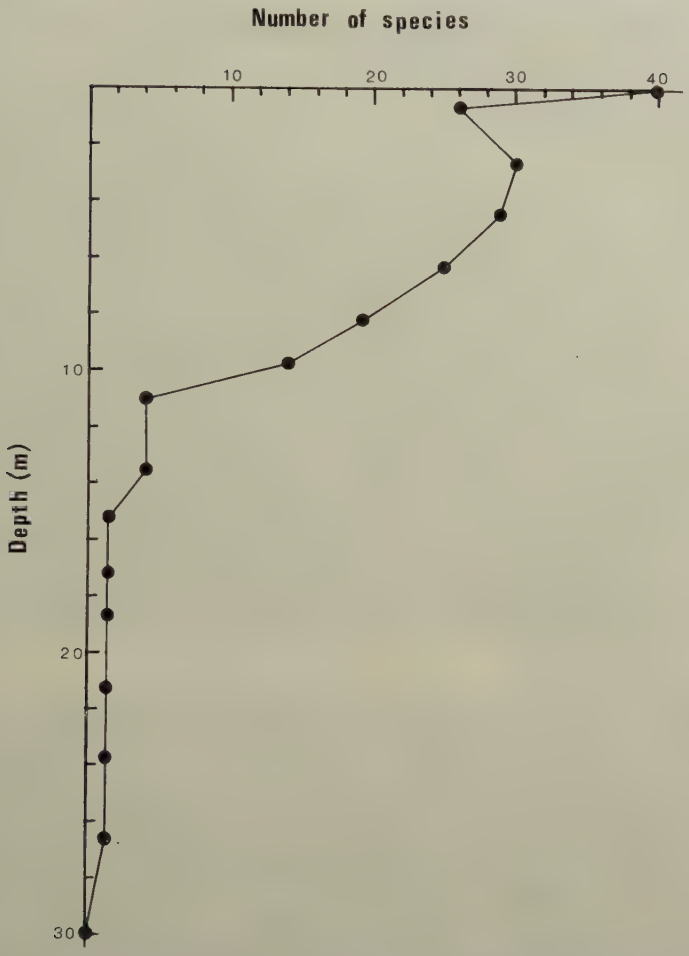



Fig. 1. Species reduction curve with depth: transect off Little Cumbrae Island, June 1976 (see Table I).

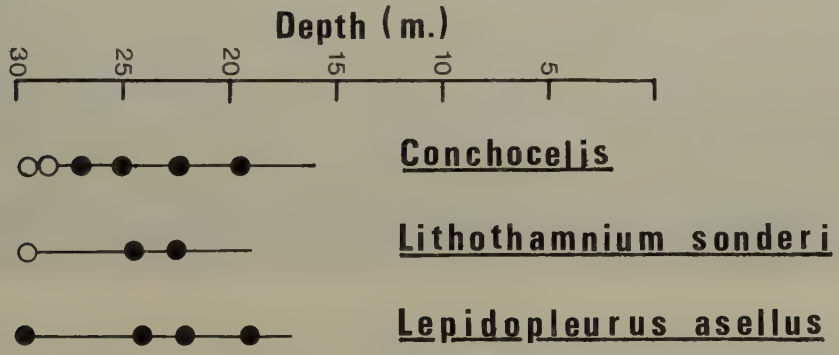
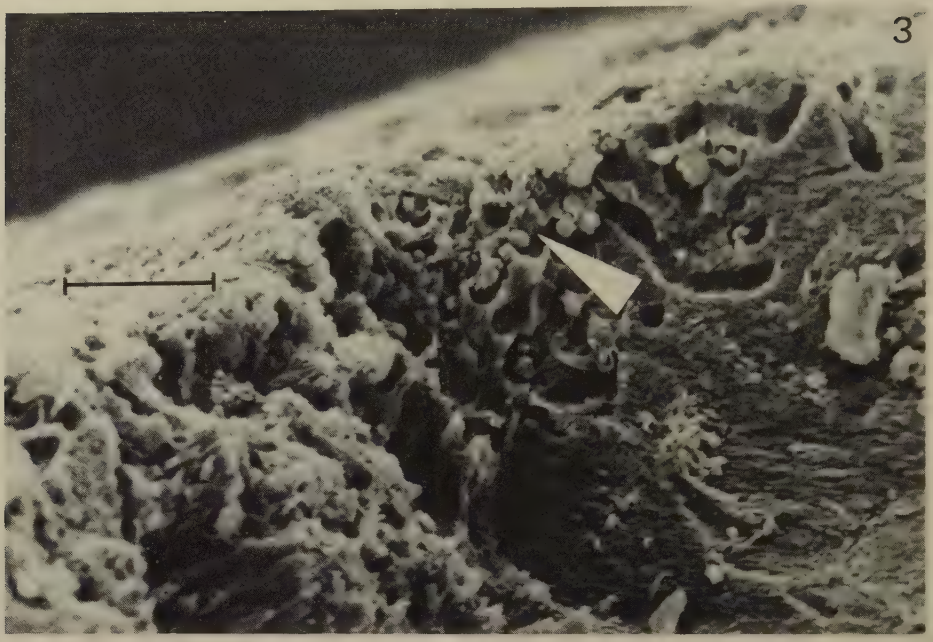



Fig. 2. Field determination of lower limit of *Conchocelis* and accompanying organisms: ●, deepest positive occurrence; ○, shallowest negative occurrence.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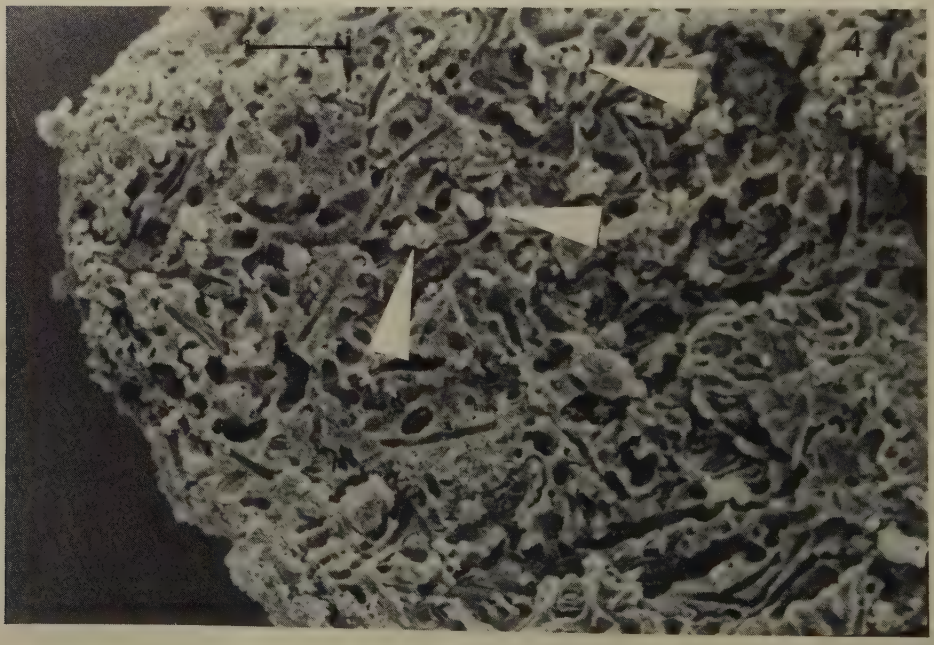
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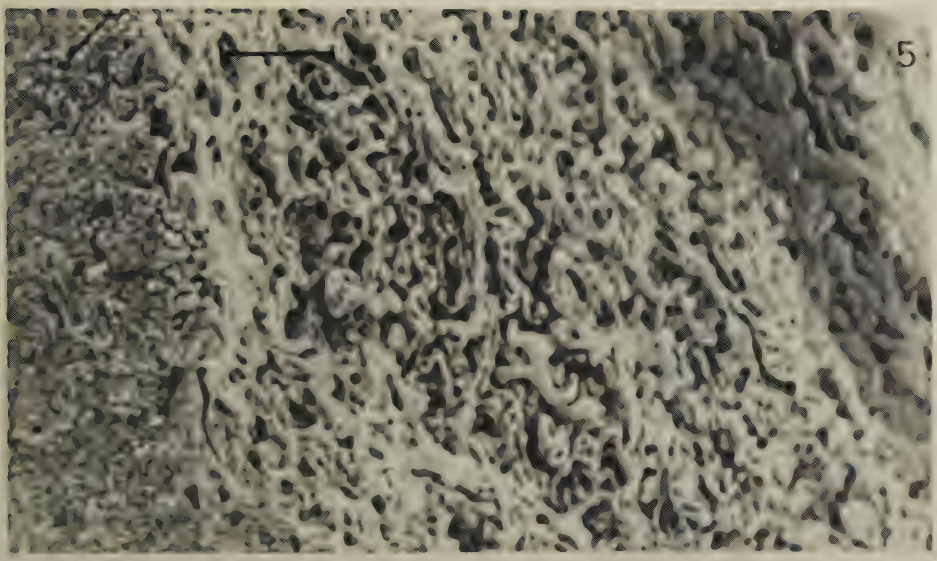

Figs. 3–7. Boring activities of *Conchocelis* in calcareous *Pomatoceros* tubes at Clashfarland Point, Great Cumbrae: Scale bars = 20 $\mu$m.

Fig. 3. 2 m depth: shallow upper surface penetration of substratum by *Conchocelis* (arrowed); other borings probably by blue-green algae.

Fig. 4. 6 m depth: boring action of *Conchocelis*, showing extensive penetration of calcareous matrix; emergent filaments arrowed.

Fig. 5. 9 m depth: similar erosion but less net-like growth.

Fig. 6. 10 m depth: superficial inorganic incrustation and marked reduction in depth of penetration by *Conchocelis*.

Fig. 7. 16 m depth: no penetration by shell-boring filaments.

The growth of *Conchocelis* in calcareous tubes of *Pomatoceros* sp. was examined after an interval of 18 months. Newly formed *Pomatoceros* tubes on an artificial reef at Clashfarland Point on Great Cumbrae were used as substrata to prevent

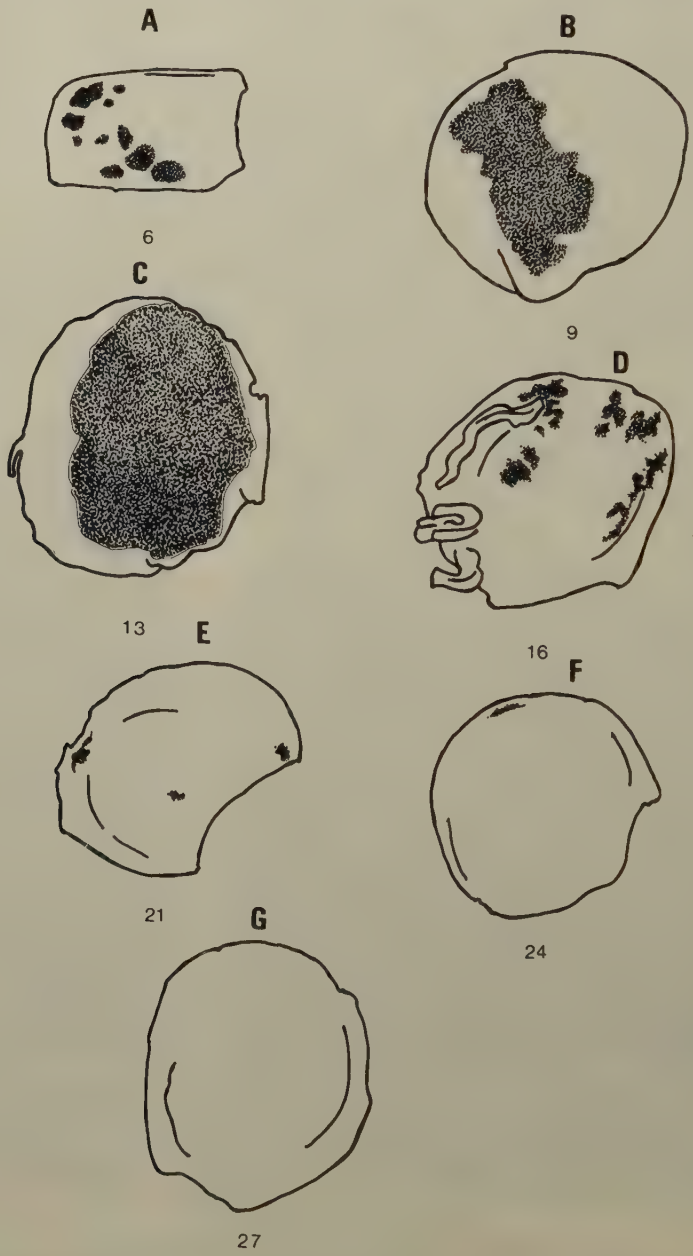


Fig. 8. Degrees of shell "infection" by *Conchocelis* with increasing depth (m): descriptions as in text.

confusion with any earlier penetrations or dead material. Here there was a much shallower cut-off point than that observed with the Little Cumbrae transect. A comparison of the shell-boring activity of *Conchocelis* in worm tubes from different depth samples can be seen in Figs. 3–7. The maximum shell-boring activity of *Conchocelis* was observed at intermediate depths (6 m), with a marked reduction in its boring activity in calcareous material at 10 m, and no *Conchocelis* at 16 m.

The distribution of *Conchocelis* filaments in shells thus shows major variations depending on the sampling depth (Fig. 8). Near the sublittoral fringe the filaments tend to be confined to the upper surface (relative to shell position on the sea bed), due to competitive effects of other organisms settled on the shell surface (Fig. 8A). In the middle range the shell material is riddled with *Conchocelis* filaments (Fig. 8B, C), whereas nearer the lower limit only the superficial shell portions appear utilized (Fig. 8D–F). Conchosporangia formation and spore production by *Conchocelis* is also maximal in the middle range of its depth distribution. The lower limits of *Conchocelis* growth in shells from a number of stations in the Firth of Clyde are

Fig. 9. Lower limits of *Conchocelis* in shells from stations in the Firth of Clyde and Clyde Estuary: C, Great Cumbrae; E, Estuary.

shown in Fig. 9. These clearly indicate the shallow nature of its depth distribution in the region of the Estuary, and the increasing depth range as one progresses from the inner to the outer regions of the Firth. This difference in depth limits between the Estuary and the deeper waters of the Firth is also seen in Fig. 10, which summarizes the lower limits of *Conchocelis* distribution at various stations in the Clyde Estuary and Firth, using the Weir in Glasgow as the starting point.

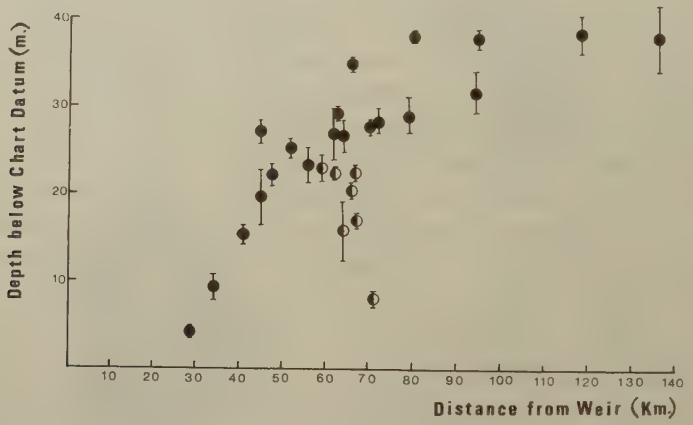


Fig. 10. Lower limits of *Conchocelis* in shells: transect (km) measured from the Weir in Glasgow, traversing the Estuary and out into the inner Firth; ●, main transect data passing between Great Cumbrae and Bute; ◐, lower limits from stations in region of the Largs-Fairlie Channel between Great Cumbrae and the mainland.

Recognition of *Conchocelis* in the field is largely a matter of experience, although the need for shell decalcification and microscopic examination is often necessary. The assumption that a pink colour to the shell is necessarily *Conchocelis* can be erroneous. Certain shells have a natural pink colouration which can be mistaken for *Conchocelis* on casual field inspection. These shells often occur on regions of the sea bed where *Conchocelis*-invaded shells are also to be found. Examples of mollusc shells, barnacle plates, and polychaete worm tube remains which have

TABLE II

Examples of substratum organisms which can cause misidentification of *Conchocelis* in the field due to their pinkish-red regions of colouration.

| | |
|---|---|
| *Arca tetragona* Pol. | Mollusca, Lamellibranchia |
| *Modiolus modiolus* L. | Mollusca, Lamellibranchia |
| *Pecten maximus* L. | Mollusca, Lamellibranchia |
| *Aporrhais pes-pelicani* L. | Mollusca, Gastropoda |
| *Verruca stroemia* O.F. Müller | Crustacea, Cirripedia |
| *Serpula vermicularis* L. (tubes) | Annelida, Polychaeta |
| Some Bryozoa | |

Ferruginous deposits also can appear pinkish.

this pink colouration, and which in consequence need careful examination, are listed in Table II. Similarly, some colonial Bryozoa can cause confusion, and ferruginous deposits on shells on occasions appear pinkish.

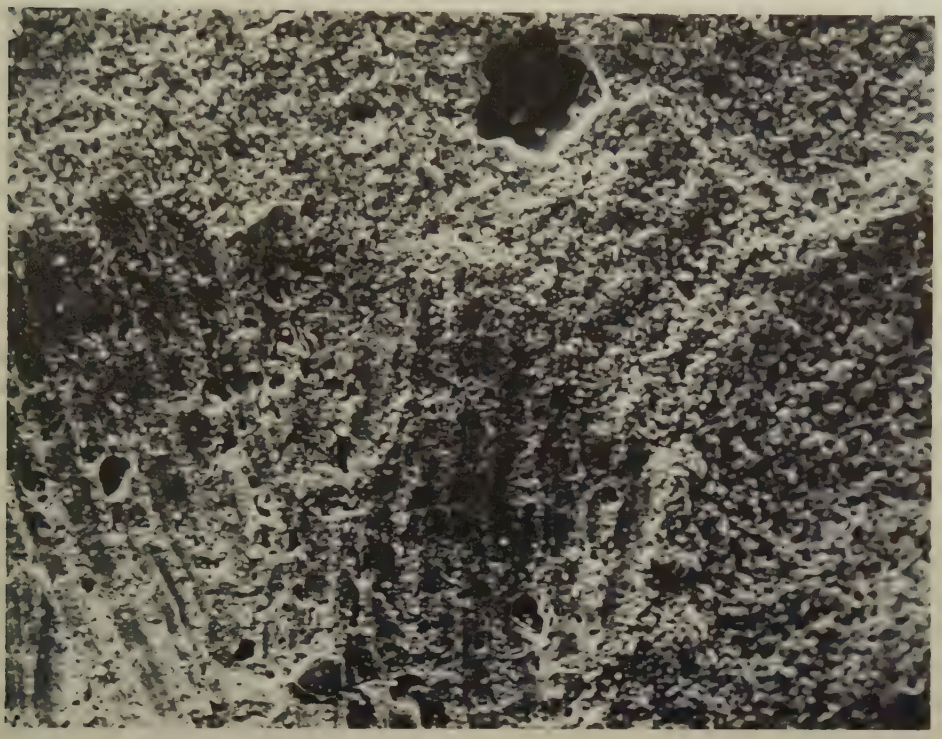

Fig. 11. Grazing action of *Acmaea* on shell with *Conchocelis* filaments: the "paths" of radula action can be seen across the *Conchocelis* bore tubes.

Another aspect of the ecology of *Conchocelis* lies in its interactions with grazing molluscs (Farrow & Clokie, 1979). Certain chitonids remove superficial particulate matter, whilst prosobranch molluscs (e.g. *Acmaea*) rasp away the shell surface by their feeding actions, and in doing so remove *Conchocelis*. This effect may be seen in Fig. 11 in which both the "tracks" of *Acmaea* radula activity and the perforations due to *Conchocelis* are observed with the filaments often in the direct feeding "path" of the mollusc. Furthermore, any organic or inorganic deposit found on the shell surface will have its effect in reducing *Conchocelis* growth, and some silt removing molluscs can also cut deep into the shell, at the same time removing layers of manganese compounds and ferruginous deposits. Whilst the removal of the silt cover and the inorganic deposits would be to the advantage of *Conchocelis*, the radula action by cutting deep into the shell will also remove some of the algal filaments, so that grazing pressures would seem to exist in these deep-water environments.

## Discussion

The contention that the lower limit of *Conchocelis* growth can be taken as coincident with the photic limit may well be considered something of an assertion in the absence of data on radiation attenuation for the same sites. It may also be argued that the acceptance of the 1% surface irradiation standard as the depth of the photic zone is very much an assumption in terms of the varied light requirements and pigment arrays exhibited by sublittoral benthic marine algae. In practical terms the data we have obtained using the *Conchocelis* distribution for the Firth of Clyde have been consistent and have enabled a photic limit diagram for the Firth of Clyde to be constructed (cf. Fig. 8). Recognition of *Conchocelis* as a participant in a standard monitoring exercise would be of considerable value in environmental impact studies where silt loading of the sea is the cause for concern. The abundance of *Conchocelis* would enable this exercise to be an annual one. Data of this nature are particularly relevant to current studies on shoreline industrialization in the Firth of Clyde with the development of the iron-ore unloading terminal in the Hunterston area. Accidental spillage of ore dust or of coal dust, or the wind winnowing of stored material, may have environmental consequences in terms of increased silt loading of the sea. *Conchocelis* thus offers a living monitoring system distributed abundantly in the Firth. One important question remains unanswered. Is *Conchocelis* capable of dark growth and the assimilation of dissolved organic compounds? If so, then the question of its reliability as a photic limit indicator remains open. The growth of certain red algae in axenic culture is stimulated by additions of vitamins and other organic compounds (Fries, 1961; see also summary in Provasoli & Carlucci, 1974) although evidence for heterotrophy appears lacking (Droop, 1974). If experimental evidence of heterotrophy by *Conchocelis* in axenic culture were to become available, it would not need to preclude the significance of its distribution in terms of photic limits as long as the normal pigment array were present. Its metabolic flexibility may be such that photosynthesis may be replaced by heterotrophy if there is protracted very dim underwater illumination or a dark period. Long term storage (4 months) in deep shade in laboratory tanks in the course of the present work has in no way affected the viability of the shell-boring filaments collected from deep water in the Firth. It is possible that short-term but efficient utilization of available radiation by *Conchocelis* may adequately balance longer periods in very dim light or darkness when it remains in a "steady-state" situation metabolically. The observations of Frankzisket (1968) and Halldal (1968) on the endozoic algae of reef corals, and of Rooney & Perkins (1972) on boring organisms in calcareous substrata of tropical seas, are especially relevant. Coral reef endozoic algae can become light-saturated at 10 lx and show photo-inhibition at 500 lx (Frankzisket, 1968). Extreme adaptations to low light intensities by algae in the giant coral *Favia* include the possession of unusually high quantities of chlorophyll $a_{720}$ (Halldal, 1968). Rooney & Perkins (1972) found that fungi were the sole boring organisms at the greatest

depths sampled, with *Conchocelis* and *Ostroeobium* as the deepest growing algae. The adaptations to very low light intensities of algae in corals could well be applied to *Conchocelis*. Surface sea temperatures in the Firth of Clyde show an annual range between 7–14 °C. Ambient temperatures of sea-bed *Conchocelis* will thus be much lower than the experimental temperature of 20 °C used with *Porphyra leucosticta* by Sheath *et al.* (1977). The lowered respiratory rates of Firth of Clyde *Conchocelis* would also bring about a lowering of light compensation points. In its utilization for monitoring purposes, problems of precise identification remain, and the relationship between underwater sea-bed topography and the cline in light penetration.

In the Firth of Clyde, *Conchocelis* only is found in shell remains in deeper water. Yet it might seem odd that a plant which bores into the calcareous shell substratum and hence is enclosed in a medium which will effect some selection of light penetration, should in addition be found at the lowest sublittoral limits of plant growth. Use of *Lithothamnium sonderi*, a species which can, on occasions, be found at similar depths, might well appear a suitable indicator organism. It may be that the deep survival of *Conchocelis* is because of its shell-boring habit, and not in spite of it. A delicate balance seems to exist between the actions of superficial grazing molluscs and the protection afforded by the shell substratum – a protection against grazers that *Lithothamnium sonderi* does not have. This grazing habit, particularly of chitonids, keeps the shell free from surface incrustations (organic and inorganic) which might further obscure light penetration. In consequence, new cleaned surfaces which will enable light transmission are constantly produced. Despite the rasping action of the radulae on the shell substance and the consequent loss of plant material, the three-dimensional filament network would clearly be advantageous in enabling new growth to compensate for removal.

Some new aspects of the biology of *Conchocelis* obtained in the course of the present work are summarized diagrammatically in Fig. 12. Maximum filament formation, shell infection and spore production occurs in the middle range of its depth distribution. Competition with both superficial deposits and other boring algae is greater just below the sublittoral fringe, and is almost gone at greater depths. Competition with sedentary animals is greater in the middle range, notably with the sponge *Cliona celata* Grant, and with polychaete worm tubes. Ferruginous deposits on the shell surfaces are of greater significance in the upper region of its depth range, whereas deposits of manganese compounds are important in the lower half of the depth range. In the shallower waters below the sublittoral fringe shell movements and change of position may be important, whereas in the deeper water the degree of superficial silt loading will be significant. Finally, grazing effects due to molluscs will result from numerous feeding organisms in the shallower water, with *Acmaea virginea* Müller becoming the more prominent grazer in the middle regions, and the chiton *Lepidopleurus asellus* from the middle region down to the lowermost limit of *Conchocelis* distribution.

The emphasis on sublittoral *Conchocelis* distribution in the present work should not detract attention from its wide environmental range. Its occurrence in barnacle shells in intertidal habitats is well known and it is apparent that the *Conchocelis* of some *Porphyra* species are equally capable of tolerating the environmental stresses associated with eulittoral habitats (Boney, 1978). In our descriptions of *Conchocelis* in the present work we have used it as a "form range" organism. The links

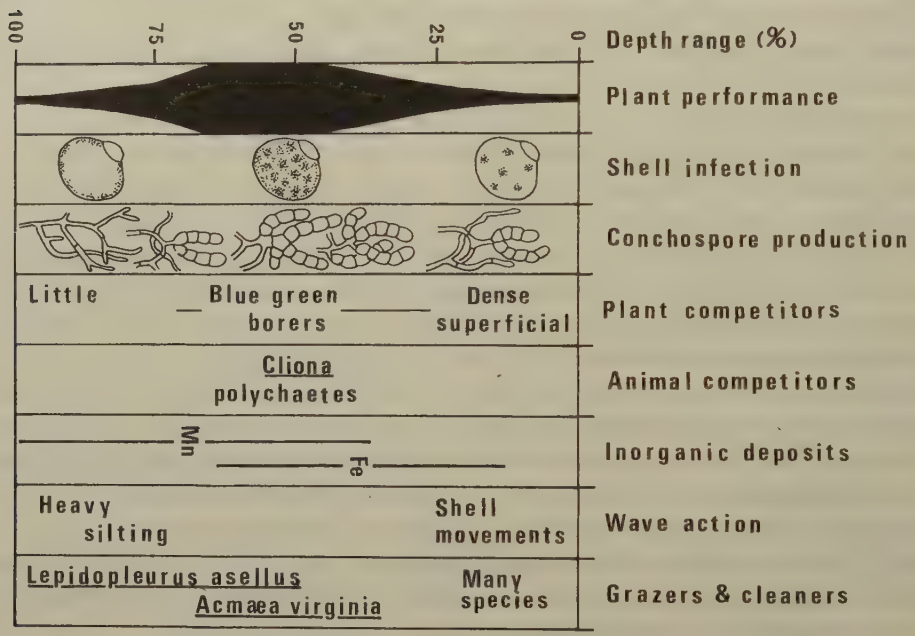


Fig. 12. Summary diagram of aspects of the biology of *Conchocelis* (see text).

between depth distributions and the associated *Porphyra* or *Bangia* phases would be an interesting correlation exercise, but one which would require culture studies under carefully controlled environmental conditions. The present work further underlines the viewpoint that in *Conchocelis* we have a thoroughly "opportunist" phase in the *Porphyra* or *Bangia* life history (Boney, 1978).

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